THE
JOURNAL OF
COMPARATIVE PATHOLOGY
AND
THERAPEUTICS.

Vol. XLVII.—No. 3. SEPTEMBER 29th, 1934. PRICE 4s.

"IMMUNE" AND "HYPERIMMUNE" CATTLE PLAGUE ANTISERUM.

By S. C. J. BENNETT, D.SC., M.R.C.V.S.

Veterinary Research Officer, Sudan Government.

THE relative potency of cattle plague antiserum obtained from immune (*i.e.*, recovered from an attack of the disease), and from hyperimmunised, cattle has been a subject for discussion since the earliest times in which such antiserum has been prepared. "Immune" serum was the first to be used; this was rapidly succeeded by "hyperimmune" serum which enjoyed undisputed favour for many years, and recently the claims of "immune" serum have again been pressed with some vigour although with very mixed approval. Any further contribution to the subject would be frankly superfluous if it merely added to the existing equivocal data without at least attempting to view the problem from some standpoint more likely to lead to its solution than those already adopted. This paper, therefore, will discuss not only the work recorded therein—which is relatively small in amount—but also the recent literature relevant to the subject, which was studied with some care in order to find an explanation for some surprising experimental results. It will be seen that by some readjustment of existing conceptions of "immune" serum such apparently anomalous results can be explained.

It is not proposed, before recording one's own experiments, to make a preliminary review of the literature, since this will be sufficiently considered in the subsequent discussion. It is, however, necessary to outline the arguments in favour of the two types of serum.

The argument for "hyperimmune" serum is that, whether or not "immune" serum may be useful, a more constantly protective product can be prepared by hyperimmunising. Furthermore, serum obtained from recovered cattle, even if initially valuable, loses its potency so rapidly that hyperimmunisation soon becomes necessary

in order to restore its titre. It is further claimed that, by suitable adjustment of the size and frequency of the hyperimmunising injections, the serum producers will continue to provide a high quality serum for long periods. This last claim will receive no attention in the present paper.

The argument for " immune " serum, which has in recent years been most forcibly presented by Edwards (1925, 1926), is that when a susceptible bovine develops an attack of cattle plague a maximal diffusion of virus (antigen) occurs within the animal's body with consequent maximal production of antibodies. It is further argued that an artificially induced attack of cattle plague, partially controlled by the injection of a small quantity of antiserum simultaneously with the virus, will serve the same purpose as a natural attack provided the amount of antiserum given is so small that it permits of the development of appreciable symptoms.

The next stage in the argument is that subsequent injections of virus into a recovered beast cannot again introduce as much antigen as was circulating during the initial attack of the disease, and in consequence such injections will be followed by a smaller antibody response. It is, in fact, argued that the term " hyperimmunisation," when applied to repeated injections of cattle plague virus into recovered cattle, is a misnomer, since true hyperimmunisation consists in treating a serum producer with progressively increasing doses of antigen, whereas in this instance, the later doses are smaller than the first.

The final stage of the argument is that, in virtue of the foregoing factors, hyperimmunisation (so-called) will not only fail to resuscitate the production of cattle plague antibodies but will not even prevent a progressive diminution in titre.

Edwards (*l.c.*, 1926) claims to have tested the foregoing theory and to have reduced its application to more or less absolute terms. He says that in the case of cattle, " the period (after the initial attack) during which the presence of antibodies in the circulating blood can be well demonstrated is a fleeting one, not exceeding 2 or 3 weeks at most, *even with the application of routine hyperimmunisation.*"* When using buffaloes the antiserum following an initial attack of the disease remains of adequate potency for at least 10 weeks.

All features of this hypothesis have not received attention elsewhere. In Africa as a whole the comparison between cattle and buffaloes as serum producers has been ruled out owing to the unavailability of buffaloes. Also no trouble has been taken to formulate critical experiments designed to confirm or refute the condemnation of hyperimmunisation quoted in the preceding paragraph. Hyperimmune serum has been almost universally used in African territories for many years, and the serum-producing cattle employed in the process have usually been subjected to repeated hyperimmunisations and bleedings over long periods (several months

*Italics not in original.

or even some years) ; the fact that their serum remains for such long periods of adequate potency, as shown both by laboratory tests and in field operations, has been accepted as sufficient proof of the value of hyperimmunisation. The preparation of immune serum has, however, recently been subjected to appreciable study, but before examining such studies it is necessary to describe one's own work, in order to discuss it together with the remainder.

Experiments on Immune Serum.

The Sudan did not, on account of various local circumstances, produce its own cattle plague antiserum until 1928. When the writer in that year undertook to investigate its practicability many difficulties were expected, not the least of which, on account of the enzooticity of cattle plague throughout most of the country, was a possible inability to obtain regular and adequate supplies of susceptible cattle for use as virus producers. Any procedure that would reduce one's requirement of these would be very valuable, and it was thought that by obtaining one bleeding, if no more, of immune serum from destined serum producers, one's requirement of virus producers would be sensibly reduced ; furthermore, by eliminating a certain amount of hyperimmunisation the process would be very desirable on both surgical and humanitarian grounds.

At the beginning of the season, therefore, all destined serum producers were given a small dose, actually 5 c.c./100 lbs. of (imported) serum simultaneously with a trace of virulent cattle plague blood. Although so little as mere marked pyrexia had been claimed as sufficient reaction for a good antibody response to ensue, it was nevertheless decided that in one's own experiment some additional clinical symptom, *e.g.*, catarrh, stomatitis, mild diarrhœa, must develop in cattle selected to provide such serum. The 5 c.c./100 lb. dose of antiserum was in fact selected in the expectation that several cattle would develop clinical symptoms and probably a few deaths would occur. The outcome of preliminary immunisation was :—

Marked reactors, for " immune " serum ..	14
Mild or non-reactors, for hyperimmunisation ..	57
Died of cattle plague	4

The marked reactors were bled on the twenty-first day (8 c.c./1 lb. body weight) and their serum was pooled and stored for subsequent testing. They were all ultimately hyperimmunised, as were the mild and non-reactors, but their serum was not mixed with that of the latter until the potency tests shortly to be described were completed. The cattle destined to produce hyperimmune serum were given intramuscular injections of citrated blood at intervals of 3 weeks. Successive doses were of 1·5 c.c., 2·0 c.c. and 2·5 c.c. per 1 lb. body weight, the last named being the final maximum, and after each injection bleedings for serum were practised on the eighth and eleventh days. Pooled serum recovered after each of the first three injections was separately stored.

A potency test was later performed on the four specially stored samples, the result of which is shown in Table I.

TABLE I.

30.4.28. Potency Test of Immune Serum, and Serum recovered after One, Two and Three Hyperimmunisations.

Serum.	*Dosage per 100 lb.*	*Bull.*	*Symptoms.*	*Result.*
Immune	5 c.c.	115	Severe cattle plague	Died
		116	Marked fever, catarrh, ulceration of mouth, but no diarrhœa. No condition lost	Survived
		117	As No. 116, but lost considerable condition	Survived
	10 c.c.	118	Marked fever 3rd—10th day. Catarrh	Survived
		119	Marked fever 3rd—10th day only	Survived
		120	Severe cattle plague	Died
	15 c.c.	121	Marked fever 3rd—8th day	Survived
		122	Marked fever 3rd—11th day	Survived
		123	Slight fever 3rd—9th day	Survived
First Hyper-immune	5 c.c.	124	Fever 4th—10th day, catarrh 9th day	Survived
		125	Fever 4th—10th day only	Survived
		126	Fever 4th—11th day only	Survived
	10 c.c.	127	No symptoms	Survived
		128	Slight fever 4th—8th days only	Survived
		129	? Thermal reaction	Survived
	15 c.c.	130	No symptoms	Survived
		131	No symptoms	Survived
		132	No symptoms	Survived
Second Hyper-immune	5 c.c.	133	Fever 4th—10th days. Few mouth vesicles 10th day, healed by 13th day	Survived
		134	Exactly as No. 133	Survived
		135	Fever 4th—10th day only	Survived
	10 c.c.	136	? Thermal reaction	Survived
		137	No symptoms	Survived
		138	Fever 4th—10th day only	Survived
	15 c.c.	139	Slight fever 5th—8th day only	Survived
		140	No symptoms	Survived
		141	Slight fever 5th—8th day only	Survived
Third Hyper-immune	5 c.c.	142	Fever 4th—10th day. Few mouth vesicles 9th day, healed by 12th day	Survived
		143	Exactly as No. 142	Survived
		144	Severe cattle plague	Died
	10 c.c.	145	Slight fever 5th—10th day. Catarrh 10th day	Survived
		146	? Thermal reaction	Survived
		147	Slight fever 6th—9th day	Survived
	15 c.c.	148	No symptoms	Survived
		149	No symptoms	Survived
		150	No symptoms	Survived
Controls		160		
		161		
		162	All severe cattle plague	All died
		163		
		164		

The data set out in Table I call for little discussion. The general conclusion is indisputable ; the immune serum was fairly good, but it was not so good as the hyperimmune samples. From a practical standpoint, however, it is not sufficient merely to view the test as a whole ; it is necessary to concentrate in particular on the dosage at which a serum would be used in field practice—*i.e.*, 10 c.c./100 lb. At this dosage the immune serum was not certainly protective against death, whereas the hyperimmune samples in all cases prevented the development of anything more than mild fever.

A secondary observation that is of some interest but of no essential importance in this particular experiment is that no appreciable difference could be noticed in the relative value of the three hyperimmune samples. It could conceivably be argued that the third was the least potent, but this deduction would be based on the death of Bull No. 144. It must be pointed out that this bull only received serum at the rate of 5 c.c./100 lb., and at this dosage no cattle plague antiserum can be expected *invariably* to protect against death.

A final assessment of the outcome of the test was as follows :—

1. Sufficient cattle had been employed, both in making and in testing the sera, for the result to be accepted as more or less definitive.

2. On this occasion immune serum had shown itself not to be quite so good as hyperimmune. It was, however, fairly good, and if one would not willingly issue it alone for field use it was at any rate fit to be incorporated in one's bulk mixed product. One was even prepared to admit that a repetition of the experiment might possibly show immune serum to be quite equal to, or even a shade better than, hyperimmune—although one frankly considered the latter possibility to be rather remote. For practical purposes, in fact, preliminary immunisation accompanied by a good " reaction " was provisionally accepted as more or less equivalent to a first hyperimmunisation ; by slightly reducing the demand for susceptible virus producers and by eliminating a certain amount of hyperimmunisation it would be economically and æsthetically desirable.

In the following season (1929) the procedure was repeated on a slightly larger scale. The intention was that, if this season's observations confirmed those of the preceding one, immune serum need not be separately stored and tested but could from the first be incorporated in the bulk stock for issue—subject, of course, to the usual potency test of the final mixture.

The outcome of preliminary immunisation was as follows :—

Marked reactors, for " immune " serum ..	27
Mild or non-reactors, for hyperimmunisation ..	128
Died of cattle plague	6

Several hyperimmunisations were carried out on the cattle which produced the purely hyperimmune serum, but serum recovered after the first three only was separately stored and tested. One minor variation in technique was introduced, namely, in virtue of having more time at one's disposal the interval between hyperimmunisations

was increased to 25 days. This minor modification can have been of no possible significance within the terms of the experiment, but it is recorded for the sake of completeness.

The results of the serum potency test, which was carried out exactly as on the earlier occasion, are set out in Table II.

TABLE II.

17.4.29. Potency Test of "Immune" Serum, and Serum Recovered after One, Two and Three Hyperimmunisations.

Serum.	*Dosage per 100 lb.*	*Bull.*	*Symptoms.*	*Result.*
Immune	5 c.c.	220	Severe cattle plague	Died
		222	Severe cattle plague	Died
		226	Severe cattle plague	Survived
	10 c.c.	205	Severe cattle plague	Died
		216	Severe cattle plague	Died
		229	Severe cattle plague	Died
	15 c.c.	206	Died suddenly, ? cause, 5th day	—
		208	Severe cattle plague	Died
		217	Severe cattle plague	Survived
First Hyper-Immune	5 c.c.	199	Severe cattle plague	Died
		221	Fever 5th—10th day. ? stomatitis	Survived
		227	Fever 4th—12th days. Few mouth vesicles on 8th day, healed by 11th day	Survived
	10 c.c.	219	Slight fever 5th—7th day only	Survived
		225	Slight fever 5th—11th day only	Survived
		231	Slight fever 5th—9th day only	Survived
	15 c.c.	194	No symptoms	Survived
		198	No symptoms	Survived
		223	No symptoms	Survived
Second Hyper-immune	5 c.c.	183	No symptoms	Survived
		193	Severe cattle plague	Died
		224	Severe cattle plague	Died
	10 c.c.	182	? Thermal reaction and reduced appetite	Survived
		195	No symptoms	Survived
		201	Fever 6th—8th day only	Survived
	15 c.c.	215	? Thermal reaction	Survived
		230	? Thermal reaction	Survived
		233	No symptoms	Survived
Third Hyper-immune	5 c.c.	171	Severe cattle plague	Died
		228	Slight fever and reduced appetite 4th—7th day	Survived
		232	Slight fever 4th—6th day only	Survived
	10 c.c.	197	? Thermal reaction	Survived
		235	No symptoms	Survived
		236	? Thermal reaction	Survived
	15 c.c.	174	No symptoms	Survived
		180	No symptoms	Survived
		185	No symptoms	Survived
Controls		176 178 187 209	All severe cattle plague	All died

The data in this Table, as those in the earlier one, do not *per se* call for much discussion, but the difference between the two sets of results is remarkable. Examination of Table II shows that all the hyperimmune sera at a dosage of 10 c.c./100 lb. protected against any symptoms except slight fever, being in this respect indistinguishable in potency from the samples considered in Table I, although it is possible that these sera were not *quite* so highly protective as those of the preceding year, since at the 5 c.c./100 lb. dosage they permitted more deaths to occur. It is when one compares the potencies in the case of the two immune sera that the astounding disparity is evident. It may be admitted that the hyperimmune sera shown in Table II were slightly less potent than those shown in Table I, but the immune serum in Table II was quite disproportionately worse than its predecessor. On the first occasion such serum fell little short of the hyperimmune samples, but in the second case it was frankly useless.

Having observed this great disparity in the two seasons' results, an attempt had to be made to find an explanation, and the only reasonable one that could be conceived was that in the cattle providing the immune serum in 1929 the attack of the disease occurring in the course of preliminary immunisation had been over-controlled. The experimental records, however, did not support this. Although a good febrile reaction had been postulated as sufficient reaction, nevertheless, in one's own experiment not only was this demanded, but some additional clinical symptom had to be developed. In any case for strictly comparative purposes there stood the fact that the immune serum producers in both seasons had been selected in an identical manner. The aggregate outcomes of the two series of preliminary immunisations are perhaps the best impartial proof of one's efforts to adopt an identical basis of selection ; as evidence of an attempt to produce good " reactions " there were in both cases a few deaths ; as evidence of conservative selection of " marked reactors " was the fact that relatively few were actually selected ; finally, more exact parallelism was proved by the fact that roughly the same proportion had been considered fit for selection in both instances.

The difference in potency of the two immune sera could not, therefore, be explained by a difference in degree of reaction to preliminary immunisation. Factors other than the cattle were, however, identical, and it seemed that the only alternative explanation was that the cattle actually did not react identically but that one was unable by mere observation of them to detect any difference. If this be the true explanation, a study of the literature should reveal widely divergent opinions as to the value of immune serum, at any rate in the case of such serum as is withdrawn from cattle in which the initial attack of the disease has been controlled by the administration of a small dose of antiserum. In the case of hyperimmune serum, conversely, little or no divergence of opinion should be apparent, since whatever the nature of a serum producer's response it is admitted to be clinically inappreciable, and having found that by a

certain technique one can sometimes prepare a good serum by hyperimmunisation it should, within narrow limits as to potency, always be possible to do so. The period over which with any given batch of serum producers one can repeatedly continue to do so is outside the scope of the discussion.

Experiment on Hyperimmune Serum.

A rather serious gap was detected when studying the literature relating to hyperimmune serum, in that the potency of serum of immune cattle destined for hyperimmunisation appeared not to have been tested against that of the same cattle after hyperimmunisation. A critical experiment had, therefore, to be devised in order to contend with the argument that, even if a hyperimmune serum be potent, the potency may not be due to hyperimmunisation but may represent, largely or entirely, the original titre attained during initial immunisation. The present seasonal method of preparing cattle plague antiserum in the Sudan, whereby serum producers for the ensuring season are immunised at the end of the current season, offered ideal conditions for studying this point. By a fortunate coincidence, when the experiment now to be described was actually in progress, the results of a highly definitive experiment of similar design, and, moreover, of identical result, were published by Hall (1933). This experiment will receive attention in due course.

In one's own experiment, twelve cattle were selected at random from over 200 destined serum producers which had been immunised by injection of antiserum and virus 6 months earlier. A small volume of blood was withdrawn from each beast and the resultant serum pooled and stored. They were then treated exactly as all other serum producers, being hyperimmunised by intramuscular injection of virulent cattle plague blood at what had been found for cattle of that type to be the maximum tolerated rate, viz., 2 c.c./1 lb. body weight every 25 days. It was not necessary to ascertain whether a single hyperimmunisation would raise the titre, and in any case tests in earlier seasons had shown that there was no appreciable difference in value between sera recovered after early, middle, or late hyperimmunisations. The object was merely to show whether hyperimmunisation, categorically, was useful, and the cattle were thus arbitrarily given three injections of virus before another pooled sample of serum was collected.

Five of the twelve cattle died from causes quite unconnected with serum production, but serum from the seven survivors was collected after the third and fourth hyperimmunisations. A pooled sample was then tested against the original immune sample at the single dosage of 10 c.c./100 lb. It was intended that if the result of this preliminary test were not clear-cut a further test should be carried out using a range of doses ; as will be seen from Table III, however, the preliminary test was quite sufficient.

TABLE III.

5.5.34. Test of Relative Potencies of Immune and Hyperimmune Serum from the same Group of Cattle at a Dosage of 10 c.c./100 lb.

Serum.	*Bull Number.*	*Symptoms.*	*Result.*
Immune	64	Severe cattle plague	Died
	65	Severe cattle plague	Died
	66	Severe cattle plague	Died
	67	Severe cattle plague	Died
Hyper-immune	68	Thermal reaction 6th—11th day only	Survived
	69	Thermal reaction 4th—12th day only	Survived
	70	Thermal reaction 4th—11th day only	Survived
	71	Thermal reaction 4th—10th day only	Survived
Controls	72	Severe cattle plague	Died
	73	Severe cattle plague	Died
	74	Severe cattle plague	Died

Nothing could be more clear-cut than this result. The immune serum from cattle immunised about 6 months earlier was useless, while hyperimmune serum from the same cattle protected against any symptoms beyond mild fever at a dosage of 10 c.c./100 lb.

Discussion.

It is not sufficient to discuss the experimental evidence relating to " immune " cattle plague antiserum without at the same time examining the hypothesis which claims *a priori* to demonstrate that it should be more highly protective than " hyperimmune " serum. It is, therefore, proposed first to discuss the theoretical argument and then to study the experimental evidence.

I.—Thoretical Considerations.

The first stage of the argument propounds that an attack of cattle plague allows the diffusion of a maximal amount of antigen (fully virulent cattle plague virus) within the serum producer's body, and in consequence a maximal production of antibodies ensues. This argument cannot be accepted as it stands, since it implies that antibody response is *without practical limit* proportional to the amount of antigen offered. One can concede no more than that *up to a certain point* antibody response may be proportional to antigen offered, but that beyond this point additional antigen is without effect.

The second stage of the argument automatically contradicts the first. This stage is to the effect that a partially controlled attack of cattle plague has the same stimulating effect on antibody " secretion " as a natural one. Partial control of the attack implies either a qualitative modification of the virus or a limitation of the extent to which it can multiply and diffuse within the serum producer's body. Whichever of these limitations may be chiefly at issue, there will not be the postulated maximal diffusion of unaltered virus, but it is still claimed that a maximal antibody response follows. The question

of qualitative modification may be left out of disuccsion ; it most certainly occurs both in natural and controlled attacks in which the patient recovers. On the point of quantitative exhibition of virus, however, a maximal antibody response can be explained by the modification demanded in the first stage of the argument. That is to say, if *up to a certain point* antibody response is proportional to antigen offered, it is sufficient to claim that in a suitably controlled attack the antigen offered at least reaches this point.

If this be so, the degree to which the attack is controlled will be a factor of vital importance—indeed, it will be the one critical factor which will determine whether or not the resultant antiserum will attain the desired potency. It will, in fact, be necessary, if " immune " serum is *invariably* to be of adequate potency—as it must be if it is to be used in routine field practice—to have some quite infallible criterion by which it is possible to determine that the initial attack of the disease has not been over-controlled. On purely theoretical grounds, there is no reason why such a criterion should not exist. If it does exist, the second stage of the hypothesis should argue that (*a*) if a small dose of antiserum will not over-control the initial attack, and (*b*) if the operator has at his disposal some infallible criterion by which he can assure himself of whether or not the attack has been over-controlled, then it will be possible to know in which cattle sufficient antigen has been offered, and by bleeding these an antiserum of the desired potency will be recovered.

The third stage argues that since no subsequent injection of virus can offer so much antigen as was offered in the initial attack, therefore, a lower antibody response will follow. This argument fails since it has already been shown that there is no proof that a *maximal* dose of antigen is necessary ; the fact that a partially-controlled attack may be as effective as an uncontrolled one implies that a *sufficient* dose only is required. Even if the hypothesis be accepted in principle, it cannot be *a priori* denied that hyperimmunisation offers sufficient antigen ; nothing but experiment can determine this.

On theoretical grounds, therefore, admitting the hypothesis of quantitative stimulation, there is no reason why equally potent antisera should not be recovered following either a natural attack, a partially controlled attack, or hyperimmunisation. If the antibody response is to any extent proportional to the dose of antigen, then a natural attack should be the most likely to offer a sufficient dose ; a controlled attack should offer sufficient provided the attack is not over-controlled, and hyperimmunisation should offer sufficient provided the antigen requirement is not greater than the amount that can actually be injected.

It is, finally, relevant to debate the subsidiary statement that the term " hyperimmunisation " as applied to injections of cattle plague virus into recovered cattle is a misnomer because all doses of virus are smaller than the first. This statement implies that hyperimmunisation strictly speaking consists in practising repeated injections of antigen of which the first is not the largest. It is certainly

common in most processes of hyperimmunisation to administer progressively increasing doses of antigen. This procedure, however, merely constitutes a policy of caution, since by giving large doses in the early stages the serum producer may be killed ; the object in all cases is to reach the maximum effective dose as soon as possible. Furthermore, having once attained to this dosage, it is not essential to " hyperimmunisation " that smaller ones shall not subsequently be given. The principle is to maintain or to restore the titre of the antiserum, and if small doses of antigen will do this the process will not be disqualified for the name " hyperimmunisation."

II.—Experimental Evidence.

From the theoretical discussion it may be concluded that the merits of " immune " serum will depend on the certainty with which the operator can ensure that sufficient antigen has been offered to the tissues of the serum producer. Furthermore, since original titre and not persistence thereof is the main point at issue, consideration may be limited to serum withdrawn shortly (within about a month) after initiating the attack of cattle plague. The titre must be considered wherever possible both in absolute and relative terms ; the latter will be more relevant to the theoretical aspects of the case, but an absolute titre must be determined for practical purposes. Finally, since on theoretical grounds it is not certain that immune serum obtained following controlled and uncontrolled attacks of the disease should be of equal potency, it will be necessary to discuss such sera as if they were two different products instead of, as hitherto, treating them as virtually identical.

1. *Serum from beasts recently recovered from an uncontrolled attack of cattle plague.*

It has been suggested that in cattle recovered from a natural, uncontrolled, attack of cattle plague, the titre should most probably be high. The literature confirms this suggestion. Richardson and Carmichael (1926), conclude indirectly, without actually demonstrating, that a natural attack provides a better serum than a partially-controlled one. They prepared only hyperimmune serum, but from two distinct classes of serum producers, namely, those that had recently recovered from natural cattle plague and those that had been artificially immunised. After hyperimmunisation the serum recovered from the former was much superior, a result that was attributed to recovery from the natural disease. Regarding absolute titre, a dosage of 2 c.c./100 lb. was adequate !

Hall (1928) records two experiments. In the first a pooled serum from beasts recovered from a natural attack was good ; it was not compared with any other serum but prevented any but the mildest symptoms at a dosage of 10 c.c./100 lb. In the second experiment three kinds of serum were tested as follows :—

(*i*) From cattle recovered from an uncontrolled attack and subsequently hyperimmunised.

(*ii*) From cattle recovered from an uncontrolled attack but without subsequent hyperimmunisation.

(*iii*) From cattle recovered from a partially-controlled attack.

A test at a standard dosage of 20 c.c. (weight of test cattle not stated) showed that there was little difference between the first and second samples, but the third was definitely the worst. Sufficient details are not given for the absolute titres to be made out.

Carmichael (1928) records a very comprehensive series of experiments with " immune " serum obtained from cattle which had developed symptoms of the following types :—

(*i*) " Blocked out "—*i.e.*, no reaction to serum plus virus.
(*ii*) " Normal " reaction to serum plus virus.
(*iii*) Recovery from natural attack 11 weeks earlier.
(*iv*) „ „ „ „ 7 „ „
(*v*) „ „ „ „ 4 „ „

Of these sera those from the naturally recovered cattle were superior, and of these the sample taken four weeks after recovery was the best. This sample protected at a dosage of 5 to 10 c.c./100 lb.

Walker (1930) tested the serum from one recovered virus producer against serum from two beasts that had recovered from a partially-controlled attack. His results may be quoted :—

1. " Serum of recovered virus producers (*sic*) protected against death at the 10, 15, 20 and 30 c.c. index dose respectively [*i.e.*, per 100 kg.] but not against a severe reaction at the 10 c.c. and 30 c.c. index dose (4 in experiment)."

2. " Pooled serum of two cattle actively immunised by the simultaneous method protected against death at an index dose of 10, 15, 20 and 30 c.c. respectively, but not against a severe reaction at the 30 c.c. index dose."

Curasson (1931) gives a summary (without protocols) of a test of three sera :—

(*i*) From strong reactors to serum plus virus.
(*ii*) From hyperimmunised cattle.
(*iii*) From cattle which had less than a month earlier recovered from a natural attack.

All were found to be of equal value, protecting at a dosage of 20 to 25 c.c./100 kg. One is at present particularly concerned with the third sample ; this was of adequate potency.

Thus the experimental evidence, if not of overwhelming volume, is at least consistent. Without exception it shows that serum withdrawn from cattle recently recovered from an *uncontrolled* infection is good. Where comparison has been made with other sera, whether immune or hyperimmune, it has always been of at least equal potency and has sometimes been better.

2. *Serum from beasts recovered from a partially-controlled attack of cattle plague.*

It has been conceded that immune serum obtained from cattle recently recovered from a partially-controlled attack of cattle plague should be of adequate potency provided the attack is not over-controlled, whereby it follows that the critical factor in practice will be

whether the operator can be sure that the attack has or has not been over-controlled. The protagonists of this type of immune serum claim that one can be sure, but there is no agreement as to what is the criterion by which one must judge, and indeed individual authors do not constantly favour the same one. For example, Edwards (1925) says " . . . the duration of antibody secretion appears to be a function of the *intensity of the morbid reaction** which the animal is is able to deal with effectively. With a ' blocked-out ' or nearly blocked-out reaction a potent antiserum is obtained only for about 4 weeks after the injection of the virus, whereas after a mild but decided reaction (with not more than a 5 per cent. mortality in the serum-producing buffaloes) a potent serum is obtained about a week after the commencement of apyrexia and then for a period of 8 weeks, and perhaps longer, without further treatment of the animals." Later (1926), he says " . . . an animal susceptible to rinderpest elaborates free antibodies, discoverable in its circulating blood, in proportion to the degree of reaction (*as estimated by the pyrexia**) it sets up to infection. . . ." He admits later on that it is difficult to know exactly how much serum, even of a carefully titrated " brew," one must give with the virus in order to get the right degree of " mild but decided " reaction. The most recent suggestion in that the antiserum should be given 2 days after the virus, and presumably—although this is not specifically stated—buffaloes so treated be used, without selection, as producers of immune serum. Unfortunately no details are provided of potency tests carried out on antiserum prepared by this non-selective process. Particular attention however, is drawn to the graphic summaries of tests on antisera withdrawn from buffaloes that had developed both good thermal reactions and clinical symptoms. At a dosage of 15 c.c./100 lb. (the only one tested) occasional deaths occurred in the test cattle. According to African standards this would hardly be considered a satisfactory potency. No more attention need be given to buffaloes beyond concluding that there seems to be a " rule of thumb " method by which a moderately good immune serum can be prepared from them with some constancy.

In regard to cattle, Rabagliati (1925) carried out tests on immune serum withdrawn from cattle judged suitable " as estimated by the pyrexia." Tested against his routine hyperimmune serum it proved so inferior as to be virtually useless. A dosage of 30 c.c./100 kg. of immune sera, drawn at intervals of from 2 to 5 weeks after immunisation did not protect against death, while the hyperimmune serum protected against any but mild symptoms at half this dosage.

Reference may again be made to Hall's (1928) tests. The basis on which the immune serum producers were selected is not stated but their serum was of low value. A second reference to Carmichael's (1928) work shows that serum from cattle which on preliminary

*Italics not in original.

immunisation showed " normal reactions " was not certainly protective against death at a dosage of 10 to 15 c.c./100 lb.

Kearney (1925) publishes an experiment, the first portion of which bears on this point. Two cattle were immunised with serum and virus, one of which was to be subsequently hyperimmunised and one kept as a control producer of immune serum. Before the first hyperimmunisation of the former the potency of both their sera was tested. At the first parallel pair of tests—53 days after immunisation—the better serum was from the beast which had given the *lesser* preliminary reaction.

Curasson (1931), as already mentioned, found in one definitive experiment that immune serum from strong reactors to preliminary immunisation was equal in potency to serum from naturally recovered and from hyperimmune animals. Later, however (1932), he says that in " field " serum from strong reactors to active immunisation he has observed great variations in potency.

Stewart (1931) gives summaries of extensive field observations without protocols. The general conclusion is that field serum varies greatly in potency, and that " variation is proportionate to the severity of the reaction to immunisation of the animal from which the serum is taken." Later, however (1933), he says, still without publishing any details, that " the one efficient way of ensuring really first-rate field serum is to draw it only from cattle which have a good thermal reaction, as shown by their temperature charts. Selecting serum-makers by choosing visible ractors is bad, as these may not have had a good thermal response to inoculation." The titre of what may be considered a good antiserum is not stated.

A most interesting experiment is recorded by Hall (1933). Two groups of six cattle were selected, the members of one of which consisted of cattle that had developed " well-marked clinical symptoms " to serum plus virus inoculation, while the members of the other group " did not react " to a similar inoculation. It is shown that up to a period of at least 5 weeks after immunisation there was no appreciable difference in the potencies of the antisera from the two groups ; both protected against anything but mild symptoms at a dosage of 25 c.c./100 kg. In the same publication a second experiment is recorded in which the potency of an immune serum was not related to the reaction of the serum producers, but as these had previously been vaccinated it is better not to admit the experiment as evidence.

To the foregoing data may be added the results of the two experiments summarised in Tables I and II of the present paper ; these need no further elaboration.

In assessing the evidence for immune serum recovered from beasts that have undergone a partially controlled attack of the disease it must be remembered that the point at issue is not whether a good antiserum may result ; it is admitted *a priori*, and confirmed experimentally that it may. The point is whether there is some criterion by which the operator can judge whether the serum recovered is likely to be of the desired potency.

It was evident from the observations summarised in Table II that following a partially controlled attack a potent antiserum would not invariably be obtained, even from cattle which had been very carefully selected on the basis of having developed symptoms amounting to nothing less than mild clinical cattle plague. Attempts to set up reactions of greater intensity would not be justified since even in this instance appreciable mortality occurred. The total evidence leads to even more remarkable conclusions, since it shows that immune antisera of all values from good to useless have been recovered from immunised cattle developing symptoms of all degrees of intensity. At one end of the paradoxical scale is a serum from cattle which " did not react " (Hall, 1933) but which was of adequate potency, while at the other end is one's own serum (Table II), recovered from carefully selected " marked reactors," which was useless.

The evidence as a whole appears to admit of only one interpretation. On theoretical grounds, working from the hypothesis of quantitative antigenic stimulation, it is obvious that, whether or not the serum-producing cattle in the more successful attempts to prepare potent immune serum were offered sufficient antigen, there was no criterion by which the fact could be judged. From the purely practical standpoint the conclusion is that although there is an encouraging degree of probability that immune serum obtained after a partially controlled attack of cattle plague will be of fair potency, in any particular instance the outcome will be doubtful.

3. *Serum from hyperimmunised beasts.*

The third stage of the original hypothesis argues that since no subsequent injection of virus can offer so much antigen as an attack, even a partially controlled attack, of the disease, a lower antibody response will follow such injections, and the resultant antiserum will consequently be of lower potency. The suggested modification of the hypothesis argues that since only a *sufficient* dose of antigen need be offered, the point at issue is whether in fact hyperimmunisation can offer sufficient, and this fact can only be decided by experiment. There will always be one factor in favour of hyperimmunisation, namely, the certainty with which a known dose of virus can constantly be offered. This is actually injected into an immune beast, and there is no question of allowing or partially inhibiting its development, such as arises when a trace of virus is administered to a partially protected susceptible beast.

In consideration of the proved efficacy of hyperimmune serum, which has given general satisfaction for over a quarter of a century, it might seem unnecessary further to discuss its merits. In relative terms it has already been shown in this paper to be at least as potent as any immune serum, and in absolute terms its titre is seen to be of considerable constancy. All existing records confirm this constancy ; slight variations certainly occur, as must be expected in any biological product of this nature, but it is invariably possible in the early stages of hyperimmunisation to produce a serum that will reduce the death

rate at a dosage of 5 c.c./100 lb. and will prevent all but the mildest symptoms at double this dosage. The evidence of this is so overwhelming that no attempt will be made to review it.

There is, however, one point to which further reference will not be entirely superfluous. Serum classed as hyperimmune is often—in fact, usually—prepared by hyperimmunising the serum producers so soon after their preliminary immunisation that it could be argued that their immune serum is not without influence on the titre. It might in fact be argued that hyperimmunisation alone will not produce a serum of high titre, but will supplement or maintain the potency developed as the result of preliminary immunisation. Theoretically, according to the modified hypothesis under discussion, either should be possible, although the original hypothesis argues that retrogression of the titre will occur in spite of hyperimmunisation. The two hyperimmune sera considered in Tables I and II were prepared immediately following preliminary immunisation and it might be claimed that this criticism is applicable. It was in order to meet much possible criticism that the experiment summarised in Table III was designed. This shows that hyperimmunisation will undoubtedly produce a high titre, although it is not shown that one hyperimmunising injection will do so. It is, however, not necessary to the argument that the *first* injection shall do so. Actually during the past few years hyperimmune serum in the Sudan has been prepared from cattle immunised several months before their first hyperimmunisation. A number of sera consisting almost entirely of bleedings after the first injection have, moreover, been tested and found up to the desired standard. Serum from the same cattle had not been tested before hyperimmunisation, but there is no doubt that during several months' rest the potency of their immune serum had dropped to a negligible titre.

An experiment by Hall (1933) appears to place the value of hyperimmunisation beyond doubt, even in regard to a first injection. Two groups of cattle to which reference was made in the preceding section (strong reactors and non-reactors to preliminary immunisation) were allowed to remain without further treatment until their serum had become of low value. They were then hyperimmunised, and in both cases their serum after the *first* injection protected against any but mild symptoms at a dosage of 20 c.c./100 kg.

Summary.

The merits of " immune " and " hyperimmune " cattle plague antiserum are discussed in the light of both theoretical considerations and experimental evidence. Initial titre is primarily at issue.

The original hypothesis in favour of " immune " serum depends on the assumption that antibody response in a serum producer is proportional to the amount of antigen (living unaltered virus) offered to the serum producer's tissues. From this it follows that since more virus is offered during an attack of cattle plague (when the whole body is saturated with it) than can be offered by any form of hyper-

immunising injection, therefore " immune " serum should be of higher protective value than " hyperimmune." It is further argued that not only an uncontrolled attack but a partially controlled one, provided it is not over-controlled, will produce a more potent serum than will hyperimmunisation. An extension of this hypothesis is to the effect that not only will hyperimmunisation not afford an initially high titred serum, but it will not even prevent retrogression of the titre established by suitable preliminary immunisation.

As against this hypothesis, it is argued that there is no proof that antibody response on the part of a serum producer is indefinitely proportional to the amount of antigen offered ; it is more reasonable to assume that provided a sufficient quantity is offered anything in excess of this quantity will be without effect. An uncontrolled attack of the disease should thus be most likely to afford a high titred serum, while in the cases of a partially controlled attack and of hyperimmunisation it will be for experiment to determine whether an antiserum of the desired titre results. Furthermore, in the case of a partially controlled attack the outcome will depend on the operator's ability not only to provoke, but even more essentially to recognise, that the required degree of control has not been exceeded. In the case of hyperimmunisation the outcome will be determined by whether in fact a sufficient dose of virus can be injected, since the amount which can be injected is limited on physical grounds.

Examination of the experimental evidence, both that provided in the present paper and earlier work, leads to the following conclusions :—

1. " Immune " serum recovered from cattle which have undergone an uncontrolled attack of cattle plague appears constantly to be of the highest potency.

2. " Immune " serum from cattle which have undergone a partially controlled attack of the disease is of extremely variable potency ; the potency, moreover, appears not to bear a constant relation either to the severity of the symptoms or to the intensity of any particular symptom in the destined serum producer during the process of preliminary immunisation. Good sera have even been recovered from apparent non-reactors, and useless sera from apparently severe reactors.

3. " Hyperimmune " serum is constantly of adequate potency.

4. The potency of hyperimmune serum is neither referable to persistence of residual antibodies formed during the process of preliminary immunisation nor to a mere maintenance of their titre by subsequent injections of virus ; it is directly referable to the actual hyperimmunising injection, as shown by the fact that beasts providing " immune " serum of low value will provide " hyperimmune " serum of high value after even a single hyperimmunising injection.

ACKNOWLEDGMENTS.

1. To Mr. A. B. MacIntyre, late S.V.S., for carrying out the test summarised in Table II. The immunisation and classification of the serum producers was the writer's own responsibility.

2. To Mr. J. T. R. Evans, Assistant Veterinary Research Officer, for preparing and testing the sera recorded in Table III.

References.

Carmichael, J. (1928). Anti-rinderpest serum—preliminary field research in Uganda. *Jour. Comp. Path. & Therap.* Vol. xli, p. 185.

Curasson, G. (1931). Nouvelles recherches sur la peste bovine. *Bull. Acad. Vét. de France*, Vol. iv, p. 304.

——. (1932). *La Peste Bovine.* Paris : Vigot Frères.

Edwards, J. T. (1925). *Rep. of the Impl. Bact. Lab., Muktesar, for the two years ending* 31*st March*, 1934. Calcutta : Govt. of India Central Pubn. Branch.

——. (1926). *Rep. of the Impl. Inst. of Veterinary Research, Muktesar, for year ending* 31*st March*, 1925. Calcutta : Govt. of India Central Pubn. Branch.

Hall, G. N. (1928). *Ann. Rep. Vety. Dept., Uganda*, 1927. Entebbe : Govt. Printer.

——. (1933). Investigations on Rinderpest Immunisation. *Thesis for Doctorate, Univ. of Zurich.* Zurich : H. Roth.

Kearney, W. (1925). *Ann. Rep. Vety. Dept., Northern Provinces, Nigeria*, 1925.

Rabagliati, D. S. (1925). The potency of anti-cattle plague serum. *Jour. Comp. Path. & Therap.*, Vol. xxxviii, p. 204.

Richardson, U. F., and Carmichael, J. (1926). Epizootic of rinderpest dealt with by local serum production in an outlying district of the Uganda Protectorate. *Jour. Comp. Path. & Therap.*, Vol. xxxix, p. 311.

Stewart, J. L. (1931). *Rep. Dept. Animal Health, Gold Coast Colony*, 1930-31.

——. (1933). *Ibid.*, 1932-1933.

Walker, J. (1930). *Ann. Rep. Dept. Agri., Kenya*, 1929. Nairobi : Govt. Press.

THE INCIDENCE IN GREAT BRITAIN OF *BR. ABORTUS* INFECTION IN VARIOUS DOMESTIC ANIMALS AS INDICATED BY THE AGGLUTINATION TEST

By F. W. Priestley.

Research Institute in Animal Pathology, Royal Veterinary College, London.

At the suggestion of the Contagious Bovine Abortion Committee of the Agricultural Research Council, samples of blood serum from cattle, horses, pigs and sheep have been submitted to the agglutination test for *Br. abortus* with a view to estimating the incidence in this country of infection caused by this organism.

The total numbers of sera examined were: 1,210 from cattle, 569 from horses, 517 from pigs and 348 from sheep. Most of the samples were obtained from the Metropolitan Cattle Market at Islington, London, through the courtesy of Messrs. J. R. Hayhurst and A. C. Lloyd, M.R.C.V.S. As this market receives for slaughter animals from all parts of the country it was eminently suitable for the purpose in view. Of the 1,210 cattle sera received from the market, 844 were collected from a butcher who slaughtered any consignment sent to him and the sex of these animals was not noted; the remaining 366 were collected from a butcher who slaughtered selected animals only and who was, therefore, presumably dealing with a more healthy type of beast. The 264 samples of sera from army horses were received through the kindness of Major-General W. S. Anthony, Director-General, Army Veterinary Services and with the assistance of Major G. E. Oxspring, R.A.V.C. A further series of 50 samples from horses was received from various sources near Winchester through the kindness of Mr. J. F. D. Tutt, F.R.C.V.S.

Technique.

Antigen. Each antigen was prepared from an ærobic strain of *Br. abortus*, which was grown on 5 per cent. potato-extract agar for three days at 37° C., the growth being washed off with carbolised saline, filtered through paper and standardised to an opacity equivalent to tube 6 of Brown's opacity standards set.

Most of the first batch of sera from the cattle market and the army horse sera were tested against *Br. abortus* strain 45, which is a bovine strain isolated by Sheather (1923). Of the pig sera 159 were tested against a porcine strain received from Dr. I. F. Huddleson, Michigan. The second batch of sera from the cattle market and the horse sera from Mr. Tutt were tested against a bovine strain isolated in 1931. These three strains were smooth when tested by the thermo-agglutination method of Pandit and Wilson (1932). As finally prepared, the suspensions were agglutinated to the same titre by a standard serum.

Serum Dilutions. Final dilutions ranging from 1 in 10 to 1 in 160 were prepared and the total amount of liquid in each tube was 1·0 c.c. To ascertain the end-titre, sera reacting at 1 in 80 or 1 in 160 were retested in

higher dilutions. Those reacting at 1 in 80 were retested in view of the possible occurrence of intermediate zones as described by Seddon (1915, 1919), Detre (1927) and Priestley (1931). Such zones were encountered in a few of the cattle sera but not in those of any other species.

Incubation. Tubes were kept at 37° C. in a dry incubator for 18 to 24 hours, when a reading was taken.

Recording of Results. For the purpose of this paper end-titre results only are recorded, these being the highest dilutions showing definite agglutination. No distinction is made between the various degrees of agglutination at the end-titre, and methods of interpolation were not used.

Results.

The results are summarised in Table I.

TABLE I.

Absolute and Percentage Numbers of Animals Reacting at the Various Titres.

	Cattle.				*Horses.*			*Pigs.*	*Sheep.*
Titre.	*From Abattoir 1st.*	*From Abattoir 2nd.*			*From Abattoir.*	*From Army.*	*From Winchester.*	*From Abattoir.*	*From Abattoir.*
		Cows.	*Heifers.*	*Steers.*					
Negative	428 (50·71)	68 (68·00)	46 (86·79)	188 (88·26)	91 (35·69)	103 (39·02)	26 (52·00)	501 (96·91)	336 (96·55)
1 : 10	107 (12·68)	11 (11·00)	3 (5·66)	18 (8·45)	77 (30·20)	106 (40·15)	18 (36·00)	15 (2·90)	11 (3·16)
1 : 20	77 (9·12)	7 (7·00)	2 (3·77)	5 (2·35)	52 (20·39)	44 (16·67)	3 (6·00)	1 (0·19)	1 (0·29)
1 : 40	47 (5·57)		1 (1·89)	1 (0·47)	18 (7·06)	8 (3·03)	2 (4·00)		
1 : 80	48 (5·69)				8 (3·14)	1 (0·38)			
1 : 160	35 (4·15)	2 (2·00)			3 (1·18)	1 (0·38)			
1 : 320	42 (4·98)	2 (2·00)	1 (1·89)		1 (0·39)		1 (2·00)		
1 : 640	25 (2·96)	2 (2·00)			2 (0·78)	1 (0·38)			
1 : 1,280	19 (2·25)	2 (2·00)			1 (0·39)				
1 : 2,560	11 (1·30)	5 (5·00)		1 (0·47)	2 (0·78)				
1 : 5,120	3 (0·36)	1 (1·00)							
1 : 10,240	1 (0·12)								
1 : 20,480	1 (0·12)								
Total	844	100	53	213	255	264	50	517	348

The figures in brackets express the results as percentages of the total.

Differences in technique make it difficult to compare the results with those of other workers. As a rule, other workers have used the designations : positive, doubtful and negative. Unfortunately, there is no general agreement, even among workers using similar methods, as to what constitutes a positive, a doubtful, or a negative reaction. The chief difficulty arises with the doubtful reactions, since it may be assumed that most positive and negative sera would have given positive and negative results with any satisfactory technique.

It is now the custom in this Institute to class as positive those bovine sera showing agglutination at 1 in 40 or higher ; as positive or doubtful, according to whether the animal is pregnant or not, those showing agglutination at 1 in 20, and as negative those showing no agglutination or agglutination at 1 in 10 only. For the present purpose, reactions at 1 in 20 were regarded as doubtful since the state of pregnancy was not determined.

Bovine Sera.

Although contagious bovine abortion is very widespread, there are no reliable statistics to show its incidence in the various countries. In a large country, such as the United States, the position is made more obscure by the fact that in general the results of each worker only refer to the incidence in a limited area. It would serve no useful purpose to quote all the references on this subject. Many of the published reports, where actual figures are given, refer only to particular herds, or else the results have been compiled with some other object in view. Thus, Graham and Thorp (1930) examined 5,702 samples of bovine sera, primarily in order to compare the tube agglutination technique with Huddleson's rapid slide method ; by both methods they found nearly 20 per cent. positive, while about 6·5 per cent. were suspicious. Murray, McNutt and Purwin (1931) examined the sera of 610 cattle slaughtered in Iowa, and found that of 155 cows, 18·7 per cent. reacted, of 205 heifers, 2·4 per cent. reacted, of 175 steers 2·3 per cent. reacted, and of 75 veal calves 4·0 per cent. reacted. In 1932 the Abortion Committee of the U.S. Live Stock Sanitary Association (1932), as a result of a questionnaire sent to all States and answered by all but two, estimated that of the 56 million cattle in that country about 18 per cent. were infected with Brucella, and that 50 to 60 per cent. of the herds were affected.

In Palestine, Stuart (1925) believed that 30 per cent. of the cattle were infected. In Denmark, Holth (1930) showed that 28·4 per cent. of the 12,905 herds examined by him between 1912 and 1919 were infected, whilst of the 39,935 animals examined 8,525 (21·3 per cent.) gave positive, and 966 (2·4 per cent.) doubtful reactions. In Russia, Makkawejsky *et al* (1931) showed that of 3,255 cattle sera examined, 1,049 (32·2 per cent.) showed evidence of infection. In Holland, Büchli (1933)

believed that 7·8 per cent. of the cows abort, and that more than 40 per cent. of the herds are infected.

In this country, A. B. Orr (1931-32) examined a total of 6,350 bovine sera during the years 1927 to 1931, and found that of 6,224 cows and heifers 29·6 per cent. were positive, while of 126 bulls 14·3 per cent. were positive. It should be mentioned, however, that these were submitted for routine diagnosis and, therefore, presumably a great many of them were suspected of being infected with *Br. abortus*.

Any estimate of the incidence of infection, as judged by the use of the agglutination test, is liable to be extremely misleading owing to the extensive use of vaccines and particularly of live vaccines. At the moment, it is impossible to distinguish between natural and artificial infection, and this point must be borne in mind when the results are considered. Vaccination is carried out extensively in this country and must account for a considerable number of positive reactions.

Taking as positive a reaction at 1 in 40 or over, and as doubtful a reaction at 1 in 20, it is seen from Table I that in the first batch of cattle sera 27·5 per cent. were positive, and 9·1 per cent. were doubtful. In the second batch, which presumably contained a better class of animal, the incidence was not so high ; of 100 cows 14·0 per cent. were positive and 7·0 per cent. suspicious ; of 53 heifers, 3·8 per cent. were positive and 3·8 per cent. suspicious, while of 213 steers, 0·9 per cent. were positive, and 2·4 per cent. suspicious. The obvious inference, and this is supported by statistical methods, is that there is considerably more infection in cows than in heifers or steers.

From these results and from those of Orr, it is probable that not less than 20 per cent. of the cows in this country are infected with *Br. abortus*, though it is impossible to estimate how much of this is due to natural infection.

It was noted that of the 1,210 sera examined, 15 showed an intermediate negative zone and 32 a negative prozone. Priestley in 1931 stated that the intermediate zone generally occurs in approximately the same dilution, namely, 1 in 160 ; this, however, is not confirmed by the present results, in which the zone was found to vary in position from 1 in 20 to 1 in 640.

Equine Sera.

That horses, especially those suffering from poll evil, fistulous withers, etc., sometimes show evidence of Brucella infection is now well recognised, though statistics indicating the incidence of infection are even more vague than is the case with cattle. It is frequently stated that the sera of perfectly healthy horses rarely give any reaction when tested against a *Br. abortus* antigen. Thus Rinjard and Hilger (1928) in France examined sera from 41 healthy horses and found only two reacting in a dilution of 1 in 30 or over, while Fitch, Delez and Boyd (1930) in the

United States examined 48 apparently healthy horses and found only four reacting at 1 in 50 or over.

A number of workers have compared the incidence of reactors in healthy horses with that in horses suffering from various suppurative conditions, but there are few references in the recent literature to the examination of large numbers of sera collected at random.

In Germany, Fontaine and Lutje (1919) examined 3,419 sera and found 17 (0·5 per cent.) reacting. In Russia, Makkawejsky *et al* (1931) examined 375 horses from 14 farms and found that 75 (20·0 per cent.) of the animals reacted at 1 in 100 or over. In Holland, van der Hoeden (1930) examined 424 horses and found 170 (40·1 per cent.) reacting at 1 in 50 or over. Later, van der Hoeden (1932) reported the examination of 482 normal horses, and of these 175 (36·3 per cent.) reacted at 1 in 50 or over. The results reported from Russia and Holland are high and can scarcely be representative of the whole country.

In the following estimate of the incidence of infection in horses in this country the same end-titres have been used for defining positive, doubtful and negative categories as in cattle ; but until far more information regarding normal agglutinins in horses is available it is doubtful to what extent this is justified. If these limits are accepted, it would appear from the results of the sera from Islington that about 14·0 per cent. of the horses give evidence of infection, while in some 20 per cent. the results are suspicious. It must be remembered, however, that most of these horses would by no means have been classed as normal ; probably they were aged and worn-out, and in any case it is likely that many of them had suffered at some time from poll-evil, fistulæ, etc. Examination of the army horses and of those received from Winchester probably gives a more exact estimate ; in these it is seen that in about 4·0 per cent. the results are positive, and in about 15·0 per cent. doubtful.

Since data concerning the age and sex of the last two groups of horses were available, it was possible to determine whether there was any difference in the incidence between the two sexes, and whether there was any relationship between end-titre and age. There was no gross difference between the sera from the army horses and those received from Mr. Tutt, and they were, therefore, taken together.

To determine the influence of sex on titre, the observed numbers were arranged as in Table II, and the expected numbers calculated on the assumption that no difference between the sexes exists. For this purpose, the numbers were combined and proportions estimated from the totals. An example will make this clear. In the cells containing negative four-year-old animals there were 21 geldings and 19 mares, making a total of 40. Expressed as a proportion of 314 (the grand total) this is 0·1274. There were tested 145 geldings ; therefore, if the proportion of negative four-year-olds is the

same as in the grand total there should be $0{\cdot}1274 \times 145 = 18{\cdot}5$. Similarly, there should be 21·5 mares. To test whether the difference between observed and expected figures is significant, the X^2 test as given by Fisher (1932) was used. In this case $X^2 = 14{\cdot}968$ and for $n = 11$ we see that p is nearly 0·2 or in other words there is no significant difference between the two sexes.

TABLE II.

Numbers of Horses of Different Age and Sex Reacting at the Various Titres.

		Age in Years.			
Titre.	4	5	6–10	11–33	
Negative	21 (18·5	26 (21·2)	15 (10·6)	12 (9·2)	Geldings.
1 : 10	10 (9·7)	10 (12·9)	15 (18·5)	14 (16·2)	
1 : 20 and over	5 (5·1)	3 (3·2)	3 (6·5)	11 (13·4)	
Negative	19 (21·5)	20 (24·8)	8 (12·4)	8 (10·8)	
1 : 10	11 (11·3)	18 (15·1)	25 (21·5)	21 (18·8)	Mares.
1 : 20 and over	6 (5·9)	4 (3·8)	11 (7·5)	18 (15·6)	

The figures in brackets are the numbers expected according to the hypothesis of similarity.

$X^2 = 14{\cdot}968$. $n = 11$. p is nearly 0·2.

Since there was no significant difference between the two sexes, the observed numbers were combined and arranged as in Table III for the purpose of testing the relationship between end-titre and age. In this case the expected numbers were calculated from the marginal totals on the assumption that no relationship exists. Thus the expected number of negative four-year-olds is $\frac{129 \times 72}{314} = 29{\cdot}6$, the number of negative five-year-olds is, $\frac{129 \times 81}{314} = 33{\cdot}3$, and so on.

The difference between observation and expectation was tested by the X^2 test in the same way as before. Here $X^2 = 43{,}685$, and for $n = 10$, p is considerably less than 0·01 ; the difference, therefore, is significant, or in other words there appears to be a rise in titre with age.

TABLE III.

Reactions of Sera of Horses at Different Ages.

Titre.	*Age in Years.*						*Totals.*
	4	5	6–7	8–10	11–13	14–33	
Negative	40 (29·6)	46 (33·3)	14 (14·8)	9 (16·8)	11 (17·7)	9 (16·8)	129
1 :10	21 (28·4)	28 (32·0)	14 (14·2)	26 (16·2)	19 (17·0)	16 (16·2)	124
1 : 20 and over	11 (14·0)	7 (15·7)	8 (7·0)	6 (8·0)	13 (8·3)	16 (8·0)	61
Totals	72	81	36	41	43	41	314

The figures in brackets are the numbers expected according to the hypothesis of independence.
$X^2 = 43{\cdot}685$. $n = 10$ p is less than 0·01.

These figures are merely an indication that there is a relationship between age and end-titre. The X^2 test necessitates a certain minimum expected number in each cell of a contingency table such as the above, and this in turn has necessitated a wide grouping. It would obviously have been more satisfactory if the titre classes could have been increased. However, the indication is quite definite and would be worth confirmation on a larger series of animals. It should be noted that in Table II the expected numbers in two cases do not conform to the requirements of the X^2 test, but there are sufficient reasons for utilising the test.

It should be noted that in this series there was no indication of any relationship between end-titre and history of suppurative conditions.

Porcine Sera.

Porcine abortion is a not unimportant menace in some countries, *e.g.*, United States and Denmark. Judging from the results obtained in the United States by Weeter (1923), Hardy, Hudson and Jordan (1929), Boak and Carpenter (1930), Johnson, and Huddleson (1931), Murray, McNutt and Purwin (1931), Huddleson, Johnson and Hamann (1933) and of Feldman and Olson (1934), the condition is widespread in that country, chiefly perhaps in the Middle West. It is obvious from these results that the incidence varies considerably in different parts of the country, as is well shown by Boak and Carpenter.

Gwatkin (1931) has reported the disease in Canada, of 501 sow sera examined 3 (0·6 per cent.) were regarded as positive and 4 (0·8 per cent.) as suspicious. In Russia, Makkawejsky *et al* (1931, 1933) examined 168 swine from seven farms and found 51 (30·4 per cent.) positive ; later they examined 5,112 animals, of which 2,345 (45·9 per cent.) were positive.

By contrast, this country appears to be free from abortion due to the porcine type of *Br. abortus*. It will be noticed that out of 517 sera tested, only one reacted at a dilution above 1 in 10 and here there was only a trace of agglutination.

Ovine Sera.

Burcella infection in sheep is not common, except perhaps in certain parts of France where it is chiefly of the melitensis type (Dubois, 1931). The disease has also been reported in Greece by Ananaides and Miaoulis (1931), and in Canada by Bruce (1930). It is possible that the disease goes undiagnosed in some areas.

The results given in Table I give no indication that sheep in this country suffer from Brucella infection. In only one case was there a reaction above 1 in 10 and here it was only slight.

Summary.

1. It is estimated that in this country not less than 20 per cent. of the cows have antibodies specific to *Br. abortus* in their blood. The incidence is considerably higher in cows than in heifers or steers, as might be expected.

2. It is also estimated that in this country 5 to 15 per cent. of the horses have specific antibodies in their blood. A statistical examination of the results obtained with one series of horse sera suggests that the incidence in males and females is similar, and that the amount of antibody present in the blood increases with age.

3. There is no evidence that in this country swine or sheep suffer from *Br. abortus* infection.

References.

Ananaidés, B., and Miaoules, N. (1931). *Rev. gén. Méd. Vét.*, **40,** 721.
Boak, R. A., and Carpenter, C. M. (1930). *J. Inf. Dis.*, **46,** 425.
Bruce, E. A. (1930). *J. Am. Vet. Med. Assoc.*, **76,** 841.
Büchli, K. (1933). *Tijdschr. v. Diergeneesk.*, **60,** 125.
Detre, L. (1927). *Z. Immunitätsf.*, **51,** 205.
Dubois, C. (1931). *Rev. gén. Méd. Vét.*, **40,** 585.
Feldman, W. H., and Olson, C. (1934). *J. Inf. Dis.*, **54,** 45.
Fisher, R. A. (1932). "Statistical Methods for Research Workers." Oliver and Boyd, Edinburgh.
Fitch, C. P., Delez, A. L., and Boyd, W. L. (1930). *J. Am. Vet. Med. Assoc.*, **76,** 17.
Fontaine and Lutje. (1919). *Z. f. Veterinärkunde*, **31,** 1.
Graham, R., and Thorp, F. (1930). *J. Am. Vet. Med. Assoc.*, **76,** 652.
Gwatkin, R. (1931). *Cornell Vet.*, **21,** 77.
Hardy, A. V., Hudson, Margt. G., and Jordan, C. F. (1929). *J. Inf. Dis.*, **45,** 271.
Holth, H. (1930). Rpt. 11th Internat. Vet. Congress, **ii,** 151.
Huddleson, I. F., Johnson, H. W., and Hamann, E. E. (1933). *J. Am. Vet. Med. Assoc.*, **78,** 849.

Makkawejsky, W. N., Karkadinowsky, J. A., Michejeff, N. J., Gawriloff, A. J., and Dawydowsky, W. G. (1931). *Deuts. tierärztl. Wschr.*, **39,** 86.
Makkawejski, W. N., Karkadinowskaja, A. I., and Micheew, N. I. (1933). *Deuts. tierärztl. Wschr.*, **41,** 321.
Murray, C., McNutt, S. H., and Purwin, P. (1931). *J. Am. Vet. Med. Assoc.*, **78,** 339.
Orr, A. B. (1931-32). *Proc. Roy. Soc. Med.*, **25,** 1501.
Pandit, S. R., and Wilson, G. S. (1932). *J. Hyg.*,.**32,** 45.
Priestley, F. W. (1931). *J. Path. & Bact.*, **34,** 81.
Rinjard, P., and Hilger, A. (1928). *Bull. Acad. Vét. de France*, **1,** 272.
Seddon, H. R. (1915). *J. Comp. Path. & Therap.*, **28,** 20.
——. (1919). *Ibid.*, **32,** 1.
Sheather, A. L. (1923). *J. Comp. Path. & Therap.*, **36,** 225.
Stuart, G. (1925). *Trans. Roy. Soc. Trop. Med. & Hyg.*, **19,** 131.
U.S. Live Stock San. Assoc. (1932). *J. Am. Vet. Med. Assoc.*, **80,** 323.
van der Hoeden, J. (1930). *Tijdschr. V. Diergeneesk.*, **57,** 15.
——. (1932). *Z. f. Haust.*, **42,** 1.
Weeter, H. M. (1923). *J. Inf. Dis.*, **32,** 401.

THE ROLE OF SALMONELLA SUIPESTIFER IN THE PRODUCTION OF AN ENTERIC DISEASE INDEPENDENT OF HOG CHOLERA VIRUS*

By H. E. Biester,

Department of Veterinary Research, Iowa State College, Ames, Iowa.

In the central states of the United States where swine husbandry is carried on intensively vaccination against hog cholera has become the established procedure in controlling the disease. In certain sections having very few swine and where herds are small and widely separated preventive and quarantine measures are carried out, the use of hog cholera virus being permitted only on official state authorisation. The simultaneous vaccination procedure as practised annually in the central states effectively controls the virus disease. Following the institution of such vaccination procedure for hog cholera, practitioners and laboratory diagnosticians are still confronted with a frequently occurring enteric disease in swine in which the virus of hog cholera is not involved and which is recognised as a definite clinical and pathological entity generally referred to in the field as " necrotic enteritis." The uncomplicated enteric disease presents primarily extensive diffuse diphtheric membranes in the large intestine. This enteric disease may occur in pigs not vaccinated against hog cholera as well as in those in which immunity to hog cholera has previously been established by simultaneous vaccination. Although quite common in young pigs it also appears in subjects of more than three months of age. Those familiar with the virus disease in the field can, as a rule, differentiate between hog cholera and the uncomplicated condition termed " necrotic enteritis," although we have observed the presence of both diseases in a herd. Owing to the volume of data obtained in the studies previously reported it unfortunately was necessary to omit considerable essential data and individual herd histories.

The first three cases of " necrotic enteritis," more suitably designated infectious suipestifer enteritis, used were not vaccinated for hog cholera. Blood was filtered from these and injected into susceptible swine with negative results. This was considered a needless and costly routine procedure that would serve no useful purpose because of our ability to distinguish between the two diseases, especially in view of the fact that some herds in which this

*Submitted in response to an editorial " Swine Fever and Paratyphoid in Pigs," appearing in the *Journal of Comparative Pathology and Therapeutics*, xlvi, pt. 4, pp. 257-265, December, 1933.

enteric disease appeared were already immune to cholera. For further confirmation of the absence of hog cholera virus, in the beginning, tissues from the central nervous system were studied histologically and the findings were negative. While we are not inclined to rely solely upon the presence of " perivascular cuffing " as a means of establishing the presence of hog cholera, the absence of such histologic changes may be considered as additional evidence of the absence of hog cholera virus in the disease entity studied.

The work of King and Wilson,[1] Dinwoodie,[2] Lewis and Shope,[3] and others, as well as our own observations show that in hog cholera the white cells usually decrease to a low level numerically. Blood counts which were made on infectious suipestifer enteritis cases from the field did not reveal a decreased number of white cells. This constitutes further evidence of the absence of hog cholera virus. The following are blood counts taken at random from suipestifer enteritis pigs obtained from different herds.

Pig No. 5,980.		*Pig No.* 7,005.	
R.B.C.	7,500,000	R.B.C.	4,528,000
W.B.C.	31,000	W.B.C.	23,000
Pig No. 7,913.		*Pig No.* 6,994.	
R.B.C.	6,880,000	R.B.C.	5,424,000
W.B.C.	24,000	W.B.C.	19,600

Pig No. 7,075.

R.B.C.	7,336,000
W.B.C.	33,500

A consideration of the daily temperature readings obtained from pigs which were fed pure cultures of the *Salmonella suipestifer* isolated from single colonies on plates and transferred to agar slants prior to the inoculation of broth showed sharp rises in temperature in less than 24 hours in some instances but never later than 48 hours. Following the injection of hog cholera virus, as a rule, 96 hours elapse before similar temperature elevations are obtained. Likewise anorexia and diarrhœa appear within 48 hours following the ingestion of *Salmonella suipestifer* cultures. This is not generally true when filtered hog cholera blood is used. When it is considered (as the editorial writer realised) that virus would have been eliminated in the isolation and transfer of organisms over culture medium and that hog cholera immune swine were used for experimental feeding of suipestifer cultures during the first few years of the studies, it conclusively shows that a disease independent of hog cholera virus was produced experimentally, proving that the suipestifer organism is a distinct pathogen. These results cannot be compared (as the critic describes) with the erroneous concept previously held by some regarding the alleged disease-producing power of the suisepticus organism. Proof of the pathogenicity of *Bacillus suisepticus* for swine has not been

shown. Its pathogenic action in rabbits is not valid evidence of its pathogenicity in swine.

Further evidence that we were not dealing with a virus is found in the fact that many of the field cases submitted for study and most of the pigs used for experimental production of the disease were previously immunised against hog cholera. The following is taken from the records :—

Pig No. 6,011, *November* 27*th*, 1922.

Premises several miles north of the Research Institute.
Breed—Duroc.
Age—5 months.
Blood—
Hæmoglobin (Talquist scale) 80 per cent.
R.B.C., 7,200,000.
W.B.C., 28,000.
Urine test for occult blood negative.

All hogs of this herd were previously immunised against hog cholera. Infectious enteritis appeared on the premises in the spring of the same year. (The exact number affected was not ascertained.) Fall pigs apparently thrived except this specimen which began to manifest intermittent diarrhœa—yellowish gray semi-liquid. Subsequent to this subject's removal, isolated cases of suipestifer enteritis appeared during the fall and in the following spring larger numbers were affected. The sanitary conditions on this farm were notoriously bad and the disease has appeared very frequently in subsequent years regardless of whether or not pigs were immunised for hog cholera. When such affected pigs were brought to the Research Institute and placed on clean ground improvement nearly always resulted. The virus disease does not respond to sanitation. Pig No. 6011 from this herd presented heavy diffuse diphtheric lesions in the cæcum and colon, with concomitant inflammatory and degenerative changes but no hæmorrhagic lesions usually characterising hog cholera were noted. Button ulcers were not present. The same pathological pictures, distinct from the virus disease, were found in cases of enteritis subsequently obtained from this herd which had been vaccinated for hog cholera. To anyone who has ever observed the effect of injected filtered virus blood or autopsied a fair number of field cases of hog cholera, it is inconceivable to visualise the *post mortem* examination of over 150 animals used during the course of these and subsequent studies with complete absence of petechiæ and ecchymoses in the kidneys, bladder, lymph nodes, larynx, subcutis and serous membranes if hog cholera virus had been present. In these infectious enteritis cases gross parenchymatous hæmorrhages were absent, and it was only in the serosa of the intestines in which the entire wall was involved by the severe necrotic and inflammatory process that some cases presented very minute hæmorrhages. This is to be expected in the locality of the severe bacterial invasion and destructive processes.

The statement " It is diminished resistance on the part of the pig and not increased virulence of the *B. suipestifer* that accounts for the sudden invasion by the organism," is not entirely in accord with our findings. Our results show that repeated animal passages enhance the virulence of *Salmonella suipestifer* for swine. Lowered body resistance no doubt is an important factor in the enteric suipestifer disease. In the case of experimental feeding of cultures the toxin present in the medium no doubt aids in breaking down the resistance of the animal to the infection because the method of preparation of the broth used in growing the organism affected the dosage necessary to infect. Pigs weighing about 70 pounds were fed embryonated ascaris ova prior to the feeding of suipestifer cultures. The lesions and symptoms produced in these pigs were more pronounced than in similar subjects not fed ascaris preparations. Parasites (nematodes and protozoa) in our opinion may be capable of producing lowered resistance. The disease is usually associated with poor sanitary conditions where lots are used for swine continuously. Where a rigid system of lot rotation is used this form of enteritis is rare. This is well borne out in a project carried out by the U.S. Bureau of Animal Industry in McClain County, Illinois, in an attempt to control ascaris infestations.

On page 263 of the editorial appears, " In the first paragraph of this report the authors state that they were convinced ' that the primary cause of this disease is the *Salmonella suipestifer*.' The next following sentence is : ' Other organisms which have been incriminated by other investigators are, in our opinion, merely secondary invaders, or play no rôle at all in the etiology of the disease.' The ' other investigators ' must have included Salmon and his co-workers, but no one would have inferred from this sentence that Salmon was the real discoverer of the bacillus now put forward as the primary cause of infectious enteritis."

The paper referred to was prepared for veterinary readers who were conversant with the literature and views held prior to the publication of our paper regarding the specific enteric disease distinguishable from hog cholera as well as with Salmon's work. The " other organisms " incriminated at the time our studies were instituted were *Actinomyces necrophorus, Balantidium coli* and *Trichomonas suis*. Many observers at that time believed the *Actinomyces necrophorus* to be the primary etiological agent. Its relation to the distinct enteritis disease recognised in the field is given in the paper discussed.[4] That Salmon first associated the suipestifer organism with hog cholera appeared to us a well-known fact which did not warrant a further didactic discussion, especially since Salmon was given recognition in that the entire group (Salmonella) was named after him in the then recent bacteriological classification, the *Bacillus suipestifer* (*Bacillus choleræ suis*) becoming the *Salmonella suipestifer*. However, while stating that Salmon first associated the suipestifer organism with swine

diseases, it should be noted that a study of his work with the organism also shows that it was complicated with virus and he was not aware of the existence of a filterable virus disease and furthermore that he did not have at his disposal swine immunised against hog cholera virus as had we, thus eliminating that factor.

Plate I of Salmon's first report[5] shows enteric lesions of what appear to be typical button ulcers generally associated at some stage with the presence of hog cholera virus which injured the intestinal lymph nodules in the wall and paved the way for bacterial invaders. This type of lesion was never found in enteritis by us even in the cases of longer standing. That Salmon first described the organism is beyond question. The work is of great historical interest and may be looked upon as a stepping stone to an understanding of hog cholera and the specific enteritis described. A later report by Salmon[6] contains illustrated gross lesions which would be considered those of hog cholera by the writer and which in my opinion present an entirely different picture from that obtained by us from feeding pure cultures of *Salmonella suipestifer*. When Salmon fed pure cultures of *Salmonella suipestifer* and excluded hog cholera he produced the enteric disease which was not then recognised as a disease entity distinct from hog cholera. In our report[4] it will be noted that gross hæmorrhagic lesions of the kidneys, bladder, subcutis, lymph nodes, spleen, etc., were not present.

In Bulletin 72 (1905), of the U.S. Bureau of Animal Industry entitled "The etiology of hog cholera," by Dorset, Bolton and McBryde, is found on page 99 the first work on the pathogenicity of the suipestifer organism in which the virus was scientifically controlled in the experimentation. They state in part: "In addition we would emphasise the fact that although the filterable virus appears to have been the primary invader in those cases of acute hog cholera which we have studied we do not deny the possibility of independent disease being caused by *B. choleræ suis*. In fact it is difficult to avoid a belief in such a possibility on account of the very considerable pathogenic power for hogs exhibited by many cultures of that organism when fed or administered intravenously. If filterable virus is capable of lowering the resisting power of hogs for *B. choleræ suis*, then it is easily conceivable that other agencies may also lessen the resisting power of these animals. So it must be admitted that a disease in hogs may exist which is due to *B. choleræ suis*, and having no connection with the filterable virus found by us in the outbreaks we have studied."

The foregoing shows that the workers who excluded the virus while studying the effects of *Salmonella suipestifer* recognised the possible rôle of *Salmonella suipestifer* as a pathogen. The general adoption of hog cholera vaccination over a period of nearly 20 years has proved that a disease entity independent of hog cholera virus exists and confirms these early writings.

The editorial writer observes : " It must also be noted that presumably (though not expressly stated), this infectious enteritis was spreading by contagion in the herd or herds from which the field cases were obtained, for diseased pigs for study were obtainable in lots of four at a time." In this connection the following facts taken from the history of one herd included in the studies are of interest. During the previous year the owner lost a large number of pigs as a result of " necrotic enteritis." This year (1923), owner has only 41 pigs, all of which were immunised against hog cholera by the simultaneous method at a time when all pigs appeared in good condition. Ten pigs are kept in an old hog house and lot where trouble occurred last year (1922). Enteritis occurred in this group of 10 pigs during July, 1923, four of which were brought to the laboratory for study. On the other side of the fence, in a clean pasture, owner kept the remaining 31, all of which were in good condition. The two lots were separated by hog wire, allowing direct contact, but the disease did not spread although the smaller group all showed clinical manifestations. That more than one animal contracted the disease at about the same time is not evidence that the disease was spreading by contact in the herds but rather that all the animals in such lots were exposed to the same degree of soil contamination by bacteria and parasites, giving each pig an almost equal opportunity to develop the enteric disease in which the virus of hog cholera was not involved.

The disease is invariably associated with poor sanitation. It was difficult to keep field cases on hand because when they were placed in clean pens which were washed frequently the diarrhœa subsided and the animals recovered unless too advanced when obtained. Furthermore when sanitary measures are instituted in sick herds they usually respond in this type of enteritis. We have not known hog cholera to respond to that type of treatment.

The simultaneous hog cholera immunisation (by means of hyperimmune serum plus virus) of herds affected with infectious suipestifer enteritis is often followed by unfavourable results. When experienced practitioners encounter herds so affected they insist upon the removal of seriously affected individuals, the institution of sanitary measures, medication and the improvement of the herd before attempting hog cholera vaccination. The fact that this procedure is fruitful of good results and stops the losses constitutes further evidence that in the United States we are confronted with an enteric disease distinct from hog cholera.

In some instances cholera-immunised swine weighing about 70 pounds were fed intestines of infectious enteritis cases which resulted in the production of typical cases of diphtheric enteritis without hæmorrhagic lesions in the parenchymatous organs or serous membranes. This fact and the experimental production of the specific enteritis constitute further proof that the *Salmonella suipestifer* is capable of producing an enteric disease if the amount of infectious material taken in is adequate, which condition would

be supplied when the lots become heavily contaminated with fæcal material or by lowering the animal's resistance to allow multiplication and invasion by the suipestifer organism as well as the *Actinomyces necrophorus*.

It is my view that the enteric diseases of swine constitute a problem comparable to that in the human species where a large number of individual factors (bacterial and parasitic) has been shown to be the cause of specific forms of enteritis. Our studies up to the present indicate to us that more than one type of enteritis may occur in swine, some characterised by less pronounced changes—swine coccidiosis being an example.

It must also be recognised that some diseases present different problems in other countries. The occurrence and control of hog cholera and swine erysipelas in the United States constitute a different problem from the one on the Continent and it would be distinctly unfair and fallacious to draw conclusions from the facts as they appear in one part of the world and attempt to apply them to those of a distant land.

Our work clearly shows that the *Salmonella suipestifer* is capable of producing a pathologic enteric condition under poor sanitary conditions in the field in both hog cholera immune and susceptible swine and that it can be produced experimentally. With the lapse of time, in the presence of destroyed intestinal tissue the *Actinomyces necrophorus* assumes an important rôle, being the chief cause of the marked thickening of the wall by its progressive invasion of the deeper structures of the intestine.

REFERENCES.

[1]King, W. E., and Wilson, R. H. *Kansas State Agric. College Exp. Sta. Bull.* **171** (1910).
[2]Dinwoodie, R. *Univ. of Arkansas Agric. Exp. Sta. Bull.* 120 (1914), p. 121.
[3]Lewis, P. A., and Shope, R. E. *Jour. A.V.M.A.*, **74** (1929), No. 2, p. 145.
[4]Murray, Chas. et al. *Jour. A.V.M.A.*, **72** (1928), pp. 35-65.
[5]Report of the U.S. Bureau of Animal Industry, **2** (1885).
[6]Report No. 46, U.S. Dept. of Agric. (1889).

CANINE DISTEMPER VACCINE.

Two Experiments with Vaccine prepared by Photodynamic Inactivation of the Virus with Methylene Blue.

By Thomas F. Dempsey
and
Victor Mayer.

Laboratory of Bacteriology, Lehigh University, Bethlehem, Pa., U.S.A.

In consequence of its high morbidity and mortality rates canine distemper is one of the most important diseases of young dogs. Prior to 1906 there were two views as to the etiological factor. McGowan[1], and Torrey and Rahe[2] held the view that a bacterium closely related to *Alkaligenes bronchisepticus* was the agent. In 1906, Carre[3] first suggested a filtrable virus as the cause of the disease. This was later corroborated by Laidlaw and Dunkin [4, 5, 6, 7, 8, 9] and is to-day the generally accepted view.

Perdrau and Todd, [10, 11, 12] in 1933, prepared a vaccine by photodynamic inactivation with methylene blue which showed a high antigenic value for the ferret. With a similar vaccine the same workers attempted a few experiments of a preliminary nature on the dog.

The purpose of this paper is to record the results of two experiments on the immunisation of dogs with a technique similar to that of Perdrau and Todd. It was impossible to obtain compound-bred dogs, such as were used by Laidlaw and Dunkin in their work on canine distemper. The dogs used were between two and three months old when injected.

Fresh infective dog distemper spleen* was ground in a sterile mortar with sterile sand and a 20 per cent. suspension made with physiological salt solution. The suspension was shaken thoroughly with glass beads.

A portion of the 20 per cent. suspension was diluted to 2 per cent. with physiological salt solution. This was centrifuged at about 2,800 revolutions per minute for 60 minutes. The supernatant fluid was decanted and filtered slowly with low pressure through a Berkefeld " W " filter. Bacterial contaminants, both ærobic and anærobic, were shown to be absent by cultural methods. Stained slides proved the absence of whole spleen tissue, which is of great importance in that it might protect the virus from being inactivated.

Four days after the removal of the spleen from a distemper-infected dog, a portion of the filtrate was mixed with equal volumes of 1 : 50,000 methylene blue in physiological salt solution. The

*From the Lederle Laboratories Inc., New York, through the courtesy of Dr. N. C. Pyle.

mixture now represented a 1 per cent. suspension of the original material.

Ten cubic centimetres of the filtrate-methylene blue mixture was put into a Petri dish (9 cm. in diameter). The Petri dish cover was replaced by a carefully selected thin glass plate which was used in all irradiations. The Petri dish containing the 1 per cent. suspension was placed on a white glass plate beneath a 75 watt, 115 volt, clear Mazda lamp set at such a distance that approximately 230 foot-candles of light fell upon the material to be irradiated. The light was measured with a Western Illuminating Meter and frequent measurements were made during irradiation to check the constancy of the light. The mixing and irradiation of the filtrate and the methylene blue were carried out in the photographic dark room. After irradiation, the material was immediately placed in a light-proof container and stored in the cold.

In the first experiment dogs were injected subcutaneously with 3 c.c. of the 1 per cent. virus-methylene-blue mixtures which had been irradiated for 0, 5, 10 and 20 minutes respectively. A control animal received 2 c.c. of the original 20 per cent. suspension. The dogs receiving material irradiated for 0, 10 and 20 minutes showed no clinical distemper symptoms. However, the dog which received a vaccine irradiated for 5 minutes developed typical distemper symptoms and died. The apparent failure of the dog injected with the unirradiated mixture to develop distemper might be explained by his relatively large size compared with the others in the group. The control animal developed distemper.

These results are shown in Table I.

TABLE I.

Results of Innoculations with Irradiated 1 per cent. Virus-Methylene-Blue Suspensions.

Dog Index.	*Dose.*	*Irradiation Time.*	*Effect.*
B	3 c.c.	0 minutes	O
F	3 c.c.	5 minutes	++
C	3 c.c.	10 minutes	O
D	3 c.c.	20 minutes	O
E (Control)	2 c.c. 20 per cent. suspension		+

O No symptoms.
\+ Clinical symptoms of distemper with recovery.
++ Clinical symptoms of distemper and death.

In the subsequent immunity test, performed 30 days after the initial injection, all surviving dogs received 2 c.c. of a 20 per cent. spleen suspension. The dog which had been immunised with a mixture irradiated for 10 minutes failed to develop distemper symptoms. The remaining two dogs and the control animal for the immunity test showed typical distemper.

These results are shown in Table II.

TABLE II.

Result of the Immunity Test.

Dog Index.	*Irradiation Time of Vaccine.*	*Results of Immunity Test. 2.c.c. of 20 per cent. Suspension.*
B	0 minutes	+
C	10 minutes	O
D	20 minutes	+
J (Control)	—	++

In a second experiment the vaccine was prepared in the same manner as in the first. Virus-methylene-blue mixtures were irradiated for 5 and 10 minutes respectively. As before, animals were injected with 3 c.c. of these irradiated mixtures. A control animal was injected with 2 c.c. of the 20 per cent. suspension from which the vaccine was prepared.

Following this injection of the vaccine, of four dogs which received a vaccine irradiated for 5 minutes three developed distemper symptoms, two of which died and one recovered. Of the four dogs injected with material irradiated for 10 minutes two died. One of these developed distemper symptoms and died. The other died without showing signs of distemper. Autopsy revealed a heavily parasitised intestinal tract. The other dogs in the group showed no symptoms. The potency control animal developed characteristic symptoms and died. These results are shown in Table III.

TABLE III.

Results of Inoculations with Irradiated 1 per cent. Virus-Methylene-Blue Suspensions.

Dog Index.	*Dose.*	*Irradiation Time.*	*Effect.*
1	3 c.c.	5 minutes	++
2	3 c.c.	5 minutes	+
3	3 c.c.	5 minutes	O
4	3 c.c.	5 minutes	++
9	3 c.c.	10 minutes	Died Worms
10	3 c.c.	10 minutes	++
11	3 c.c.	10 minutes	O
12	3 c.c.	10 minutes	O
13 (Control)	2 c.c. 20 per cent. suspension	—	++

The immunity test was performed 30 days later. The two surviving members of the 5-minute group showed no symptoms of distemper. On the other hand, the two surviving members of the 10-minute group developed distemper and died. These results are shown in Table IV.

TABLE IV.

Results of Immunity Test.

Dog Index.	*Irradiation Time of Vaccine.*	*Result of Immunity Test. 2 c.c. of 20 per cent. Suspension.*
2	5 minutes	O
3	5 minutes	O
11	10 minutes	++
12	10 minutes	++
14 (Control)	—	++

Conclusions.

1. As Perdrau and Todd have indicated, suspensions of filtered canine distemper virus containing no whole spleen tissue appears to be photodynamically inactivated in the presence of methylene blue.

2. With the method of photodynamic inactivation used, the antigenic value for the dog seems to be highly sensitive to small differences in irradiation time.

3. In the two experiments the vaccines were prepared from different spleens. One showed protection with 10 minutes of irradiation, while the other showed protection after 5 minutes of irradiation.

4. Should such a vaccine prove effective, successful application depends upon a method of standardisation of the virus.

Acknowledgment.

The authors wish to express their thanks to Dr. Stanley J. Thomas for his aid and criticism.

References.

[1]McGowan, J. P. "Some Observations on the Clinical Symptoms, Prophylaxis, and Treatment of Distemper." *Vet. Jour.*, Jan., 1912.

[2]Torrey, J. C., and Rahe, A. H. "Studies in Canine Distemper." *Jour. of Med. Research*, Vol. xxvii, No. 3, Jan., 1913.

[3]Carre. *Bull. de la Société, Centr. de Méd. Vétérinaire* (Paris), 1905.

[4]Laidlaw, P. P., and Dunkin, G. W. "Dog Distemper in Ferret." *Jour. Comp. Path. & Thera.*, Sept., 1926, Vol. xxxix, 201.

[5]——. "Experimental Distemper in the Dog." *Jour. Comp. Path. & Thera.*, Sept., 1926, Vol. xxxix, 213.

[6]——. "The Nature of the Virus." *Jour. Comp. Path. & Thera.*, Sept., 1926, Vol. xxxix, 222.

[7]——. "Immunisation of Ferrets against Dog Distemper." *Jour. Comp. Path. & Thera.*, Mar., 1928, Vol. xli, 1.

[8]——. "Immunisation of Dogs." *Jour. Comp. Path. & Thera.*, Sept., 1928, Vol. xli, 209.

[9]——. "Dog Distemper Antiserum." *Jour. Comp. Path. & Thera.*, Mar., 1931, Vol. xliv, 1.

[10]Perdrau, J. R., and Todd, C. "Photodynamic Action of Methylene Blue on Bacteriophages." *Proc. Roy. Soc. B.*, **112**, 277.

[11]——. "Photodynamic Action of Methylene Blue on Certain Viruses." *Proc. Roy. Soc. B.*, **112**, 288.

[21]——. "Canine Distemper. The High Antigenic Value of the Virus after Photodynamic Inactivation by Methylene Blue." *Jour. Comp. Path. & Thera*, June, 1933, Vol. xlvi, 78.

FURTHER OBSERVATIONS ON CELL INCLUSION DISEASE OF FOWLS, AND DIFFERENTIAL DIAGNOSIS FROM FOWL PLAGUE.

By S. J. Gilbert
and
G. B. Simmins.

Veterinary Laboratory, Jaffa, Palestine.

In 1931[1] the authors described their experiments on this disease and their finding of a virus as the cause. Until a few months ago, the disease has been diagnosed on comparatively few occasions since that time and rarely in a very acute form. The irregular occurrence of an acute type of the disease was previously referred to and also the fact that native flocks in the villages were sometimes affected by this type as well as pure bred poultry.

The present outbreak, however, has been more widespread than previous ones. It cannot be said to have been more acute than those observed on previous occasions, but it has caused greater alarm on account of its occurrence over a wider area. We have previously shown that a type of the disease which was just as acute could also be produced by rapid passage from sub-acute cases.

Another interesting feature in connection with the present outbreak was the occurrence of severe mortality in three-weeks-old chicks in one case and in ducklings in another.

The disease was first observed in the Jaffa district, and cases were almost continuously reported for a period of some 6 weeks, after which time it subsided. Shortly afterwards an outbreak was reported from Jerusalem, mostly occurring in small flocks in an urban area, and which lasted for a period of a few weeks.

Inoculation experiments were performed at the laboratory from a number of sick or dead fowls forwarded for examination. The results of these experiments showed the same peculiarities as had previously been described in connection with the disease, *viz.*, the instability of preserved virus after a very short period, and the association with leucocytic inclusions. The following table shows the results of the inoculation of various material at different intervals after storage in glycerine saline for more than 10 days.

The same virulent material used in less than 6 days was invariably fatal.

Fowl Inoculated Number.	*Age of Material.*	*Dose.*	*Route.*	*Result.*
37	12 days	0·5 c.c.	Intramuscular	Lived
11	13 „	0·2 c.c.	Subcutaneously	„
12	13 „	0·2 c.c.	„	„
19	15 „	0·2 c.c.	„	„
20	15 „	0·2 c.c.	„	„
36	19 „	0·5 c.c.	Intramuscular	„
26	31 „	0·5 c.c.	„	Died 3rd day
6	15 „	0·5 c.c.	„	„ 8th „
5	22 „	0·5 c.c.	„	Lived
4	6 „	0·5 c.c.	„	„

It will be seen from the foregoing table that there were two exceptions in which the virulence was maintained for much longer than the usual period. In the case of the stored organ material which killed fowl No. 26 in 3 days, this material was inoculated 3 days later into fowl No. 29, which remained unaffected, though subsequent inoculation with virulent material killed it after 3 days.

Post-mortem Lesions.

On the whole the post-mortem appearances were similar to those previously described but petechiæ of the proventriculus were more frequently observed, approximating to some 15 to 20 per cent. of cases. Such lesions have been seen in connection with cell inclusion disease before, but only on rare occasions. These petechiæ were if anything slightly larger and more diffuse than those seen in fowl plague, but in one or two instances they were indistinguishable.

The prevalence of such lesions in fowls which had died from the normal type of fowl plague led to some confusion in the lay mind and fear of the more deadly disease, *i.e.*, plague. Fowl plague was prevalent in Palestine in 1928, but on the application of energetic control measures, including stoppage of movement, it was eradicated in less than six months. On that occasion there was every indication that the disease had been imported from Egypt although import of fowls from that country has always been prohibited. Lesions of the proventriculus are usually seen in typical plague. They are not described in connection with Newcastle disease[2], nor are petechiæ of the breast bone, the latter being frequently seen in both cell inclusion disease and plague.

Apart from petechiæ in the proventriculus, the lesions resembled those previously described. Inflammation of the bowels was of a variable nature. In a few cases this was very severe but not repeated on sub-inoculation. In others it was hardly discernible. In most of the specimens the liver was yellowish or presented diffuse areas of a yellowish tinge, and this so far has proved to be the most diagnostic lesion apart from inclusions. Petechiæ on the heart and breast bone were also usual.

Inclusion Bodies.

Inclusions in the leucocytes were found on blood examination

with the exception of two fowls which arrived in a decomposed condition, but on the first sub-passage in each case the inclusions were found.

Mortality.

The mortality in outbreaks has been very variable. It was most severe amongst small owners where the fowls are herded together in small yards.

In these cases 100 per cent. of deaths has been usual. In large flocks where ample space is provided it has been as low as 2 per cent. It has been found advisable to destroy all sick birds immediately if the mortality is to be reduced.

Dried Virus.

Some experiments were made on the maintenance of virulence in dried organ material. Liver and spleen were mashed in a mortar and spread in a thin layer on glass plates and dried over sulphuric acid. After 27 days it was mixed with saline to make the usual quantity injected, viz., 0·5 c.c. of 20 per cent. emulsion. A fowl inoculated intramuscularly with this material showed symptoms and was killed on the sixth day, and diagnosis was completed by a further passage, from which a fowl died 5 days later.

After 48 days' storage of the dried material, 1 c.c. of emulsion was inoculated intramuscularly into a fowl, which died on the seventh day, but a second fowl which received 0·5 c.c. of the same emulsion lived although clinically affected. It would appear that the virus is more resistant in the dried state.

According to Ostertag and Bugge[3] fowl plague virus was destroyed by drying in a thin layer after 39 days and by putrefaction after 39 days, whilst blood mixed with glycerine was infective up to 270 days (Maue)[3]. In cell inclusion disease putrefaction appears even more rapidly to lower the virulence of the virus or destroy it. Liver emulsion from a fowl which was received in a putrefied state from a Jerusalem outbreak was filtered and 2 c.c. of the filtrate was inoculated intramuscularly into a healthy bird, which died 11 days later. The usual quantity of the same emulsion unfiltered was inoculated into another fowl and this bird passed through a mild attack and showed numerous inclusions in the blood. By passage experiments the disease was further identified.

Virulence of the Virus.

The virulence of cell inclusion disease virus, as might be expected, was found to be also variable. On previous occasions blood had not been found to be a very certain inoculum though occasionally it was quite virulent. Spleen and liver emulsion had been found to be more certain in their effect.

In the first experiment blood was taken from a fowl suffering from an acute attack and which later succumbed after 2 days' illness. Two fowls inoculated with 0·001 and 0·00001 respectively both died on the fourth day.

The experiment was repeated by the incoulation of blood from fowl No. 9 on the fourth day after inoculation, when the temperature had fallen to 107·2°. No. 9 died on the eighth day. It had shown inclusions in the blood throughout and diagnosis was further confirmed by a fatal sub-passage. The blood from No. 9 was inoculated subcutaneously into three fowls as follows :—

No. 10—1 c.c. pure blood.

No. 11—0·01 c.c. pure blood.

No. 12—0·0001 c.c pure blood.

Nos. 10 and 11 showed increased temperature and typical inclusions. No. 10 was killed on the sixth day and diagnosis was completed by sub-inoculation into No. 14, which died 4 days later. No. 11 died on the seventh day. No. 12 has continued to live. Its temperature never rose above 107·8°, and blood films were continuously negative.

Summary.

The similarity of exceptional cases of cell inclusion disease to fowl plague raises the question of identity and differential diagnosis. There are, however, great differences between them and these differences would appear to separate them clearly.

They may be summarised as follows :—

1. Cell inclusion disease is well known to exist in a chronic form, and is sometimes so slight that, clinically, it would pass unnoticed.

2. The variable mortality even in acute outbreaks.

3. The usual instability of the virus when preserved in glycerine saline.

4. The association of the disease with distinct inclusions in the leucocytes.

5. The frequent occurrence of visible alteration in the liver, and the absence in the majority of cases of marked bowel changes.

6. The variation in virulence according to the severity of the attack.

References.

[1]Gilbert and Simmins. *Journal of Comparative Pathology & Therapeutics*, Vol. xliv, Part 3, Sept., 1931.

[2]Doyle. *Journal of Comparative Pathology & Therapeutics*, Vol. xl, Part 2, June, 1927.

[3]Cited by Hutyra and Marek, pp. 361.

EXAMINATION OF THE BRACHIAL PLEXUS OF NORMAL FOWLS FOR LYMPHOID INFILTRATIONS.

By V. Elvira Palmer and Jacob Biely.

The University of British Columbia, Vancouver, Canada.

In the course of previous work on fowl paralysis (*Neurolymphomatosis gallinarum*) it was found that typically paralysed birds showed characteristic enlargements in the dorsal root ganglia of one or the other side of the brachial plexus more often than in the lumbosacral plexus. Thus in a recent paper[2] it was reported that out of 45 paralysed chicks 86·6 per cent. showed gross lesions in the brachial plexus as compared with 69·17 per cent. in the lumbosacral plexus. Similar percentages were obtained with large numbers of birds submitted for routine post-mortem examination. It would therefore appear as if the brachial plexus constitutes a comparatively vulnerable region for the development of the gross lesions of fowl paralysis.

Simultaneously Jungherr[4] published a paper in which he confirmed our findings. In fact he states that nerve lesions are most apt to be found in the brachial plexus and that the common autopsy practice of examining the sciatic trunks only is inadequate. It may be noted, however, that although gross lesions are most frequently found in the brachial plexus the wings of birds showing these gross lesions do not show in the majority of cases external manifestations of paralysis.

In view of the common occurrence of gross lesions in the dorsal root ganglia of the brachial plexus of paralysed birds it was thought worth while for comparative purposes to make a microscopic examination for the presence of lymphoid cells in the spinal cord and nerve roots in the brachial region of apparently normal birds.

Material and Methods.

Fourteen birds which were in good condition and to all appearances perfectly normal were killed and examined. All organs were found to be normal and the digestive tracts were singularly free from parasites. The dorsal root ganglia and nerves of the brachial plexus and the spinal cord in that region were removed from each bird, fixed in Zenker's fluid, dehydrated and embedded in paraffin according to the usual technique. Serial sections (4,459 in all), were made throughout the entire length of the brachial region of each of ten of the birds. In the case of the other four birds serial sections (652) were made in each case of the cord and one pair of roots in the brachial region, and in addition sections were made of the brain and sciatic nerves. Five other normal birds from two

flocks with a negative history for fowl paralysis and two normal birds (fowl paralysis history in flock unknown) were dealt with in similar fashion. (Three of these birds showed a few round worms in the digestive tract.) All of the sections were stained in hæmatoxylin and eosin and were examined carefully for lymphoid infiltrations.

Microscopical Examination.

The microscopical examination of the brachial plexus of the fourteen birds in the first group revealed in every case dense areas situated in the vicinity of small capillaries in the dorsal root ganglia and composed mainly of small lymphoid cells. Occasionally there were present one or two medium-sized lymphoid cells or large lymphoid cells with large vesicular nuclei. In some cases the area was very tiny, while in others it occupied about one-half of a high power field ($\times$ 400). Plasma cells also occurred, usually scattered between the ganglion cells and nerve fibres. Sometimes the meningeal vessels of the spinal cord showed a slight perivascular infiltration and occasionally a small group of small lymphoid cells might be observed about a vessel in the white matter. There was no evidence of mitosis in any of the lymphoid cells, either in the brachial plexus or in the spinal cord. Since the histological picture was much the same in each case, only two birds will be described in detail to serve as illustrations.

Bird No. 3,770 (*Laboratory No.* 55), *October* 26*th*, 1933. *Weight*, 1,645 *grammes.*

Slides 1 and 2.—No lymphoid infiltrations present.

Slide 3.—Tiny area of small lymphoid cells close to edge of dorsal root ganglion.

Slide 4.—Roots and cord free from infiltration.

Slides 5 and 6.—Tiny dense area of small lymphoid cells in centre of dorsal root ganglion near capillary.

Slide 7.—Very tiny area of small lymphoid cells at edge of dorsal root ganglion.

Slide 8.—Same as 5 and 6.

Slides 9 to 15.—No infiltrations.

Slide 16.—Tiny dense area of small lymphoid cells in region of capillary at edge of dorsal root.

Slide 17.—No infiltrations.

Slide 18.—Dense area of small lymphoid cells along edge of capillary in dorsal root.

Slides 19 and 20.—No infiltrations.

Slide 21.—Tiny area of small lymphoid cells close to capillary at edge of dorsal root.

Slides 22 to 29.—No infiltrations.

Slide 30.—Tiny dense area of small lymphoid cells adjacent to small capillary in root.

Bird No. 3,501 (*Laboratory No.* 58), *October* 30*th*, 1933. *Weight*, 1,412 *grammes.*

Slide 1.—A few small lymphoid and plasma cells in surface sections of brachial root.

Slide 2.—Small dense group of small lymphoid cells near small vessel in root. Small lymphoid cells and plasma cells scattered here and there through dorsal root ganglia on both sides of cord.

Slide 3.—Tiny areas of small lymphoid cells near small vessels. Also small lymphoid cells and plasma cells scattered throughout both dorsal roots.

Slide 4.—Small areas of small lymphoid cells and a few mononuclear leucocytes near small capillaries. A few groups of plasma cells and scattered plasma and lymphoid cells throughout dorsal root on both sides of cord.

Slide 5.—Two small dense areas consisting mainly of small lymphoid cells near small vessels. A few lymphoid and plasma cells scattered about in root.

Slides 6 and 7.—Tiny area of small lymphoid cells near a small vessel and a few scattered cells as in Slide 5.

Slide 8.—Continuation of area in Slide 7. Area has become a little larger. Tiny area also occurs at edge of dorsal root on the opposite side of cord.

Slide 9.—Dense area consisting mainly of small lymphoid cells near a small capillary. Near the edge of the dorsal root there are a number of small lymphoid cells along the length of a small capillary. There are a few lymphoid cells along the other edge of the dorsal root. A few plasma cells in groups and a few scattered lymphoid cells occur.

Slide 10.—Few small lymphoid cells at edge of dorsal root near tiny vessel and a few scattered cells.

Slide 11.—Fairly large area consisting mainly of small lymphoid cells, some plasma cells, mononuclear leucocytes, and one or two polymorphonuclear eosinophiles in surface sections of dorsal root. Tiny area of small lymphoid cells near small vessel in dorsal root of opposite side.

Slides 12 and 13.—Dense areas consisting mainly of small lymphoid cells about vessels at edge of dorsal root. There are also a few lymphoid and plasma cells scattered between ganglion cells and nerve fibres.

Slide 14.—Surface section of root showing continuation of area in Slides 12 and 13.

Slide 15.—A few lymphoid cells scattered about in dorsal root.

Slide 16.—A few lymphoid cells occur at the edge of dorsal root.

Slides 17 and 18.—A small area of small lymphoid cells in vicinity of a small vessel in dorsal root. Also small lymphoid cells scattered along edge of dorsal root. There is a narrow rim of small lymphoid cells about meningeal vessels of cord.

Slide 19.—A few lymphoid cells at edge of dorsal root ganglion.

Slide 20.—A few lymphoid and plasma cells in dorsal root ganglion.

Slide 21.—A few lymphoid cells in dorsal root ganglion.

Slide 22.—A few lymphoid cells near small capillaries in dorsal root ganglion.

Slide 23.—A small area of small lymphoid cells near a tiny capillary in both dorsal root ganglia.

Slides 24 and 25.—Same as 23, and also a few scattered lymphoid cells.

Slide 26.—A small group consisting mainly of small lymphoid cells in surface sections of dorsal root. There are also a few plasma cells.

Slides 27 to 31.—No infiltrations.

The sections of brain and sciatic nerve made from four of the fourteen birds did not show any areas of lymphoid cells in three birds. In the case of the fourth bird a few tiny groups of small round cells were seen in the cerebrum. These cells varied in

size and shape and were situated near small vessels. One section showed a small dense area consisting mainly of small lymphoid cells. Some of the cells were amœboid in appearance.

Four of the five normal birds obtained from the two flocks with a negative history of fowl paralysis showed lesions in the dorsal root ganglia which resembled those described for the fourteen normal birds in every respect. In the fifth bird the lesions were larger and there was both a more pronounced infiltration of the meninges with lymphoid cells and a greater amount of perivascular lymphoid infiltration of the small vessels of the spinal cord. Most of the cells in the lesions were a little larger than small lymphoid cells. Also a great many of the cells were irregular in size and shape. Several of the large vesicular type of lymphoid cell were present. In most of the areas there were no cells in mitosis but a few areas did show one or two such cells.

The spinal cord and dorsal root ganglia of the two birds in the third group presented the same histological picture as those of the birds in the first group. The sciatic nerves did not show any lymphoid infiltration.

Discussion.

One of the chief difficulties encountered in the study of fowl paralysis is the presence of lymphoid infiltrations in apparently normal birds. Thus Pappenheimer *et al*[6 & 7] found slight lesions in the nervous system of normal birds to be of such a common occurrence that they would have been inclined to regard them as a normal histological feature if some of their material had not been entirely free from such infiltrations. In fact, these authors regard the presence of slight lesions in apparently normal birds as signifying the existence of fowl paralysis in a mild form, undisclosed by clinical symptoms. They report that their " material includes an unbroken series of transitions between the slightest lesions and those which approach true neoplasms in the intensity of the cell proliferations."

As far as the technique employed in this study reveals, the only apparent differences between the lesions in the dorsal root ganglia of the brachial plexus in unparalysed birds and those in birds showing clinical symptoms of fowl paralysis is that in the latter the characteristic lesion, besides being larger and denser, shows a decided increase in the number of medium-sized lymphoid cells and also in the number of large lymphoid cells with large vesicular nuclei. Besides this there is always a number of cells in mitosis. There is also usually a considerable number of the large, medium and small type of lymphoid cell and some plasma cells scattered among the ganglion cells and nerve fibres. But these differences are in degree of intensity only, and it would seem that a careful cytological study of the lymphoid cells found in the dorsal root ganglia of unparalysed birds and of the lymphoid cells forming the characteristic lesions in paralysed birds would be the logical

procedure in an attempt to throw some light on the significance of the slight lesions in the dorsal root ganglia of apparently normal birds. In this connection it is worth bearing in mind that Potter and Richter[8] in their studies on mouse leukemia, were faced with the question as to whether the cells of the characteristic accumulations in the inoculated animals were descendants of the living cells introduced by inoculation or of cells originating in the host. Special cytological methods were employed by these authors and it was found that none of the methods used differentiated between the cell type described for the pathological tissue and a morphologically similar type found in normal mouse tissues.

In view of the fact that our material shows that in nineteen apparently normal birds there was a constant occurrence of areas of lymphoid cells in the dorsal root ganglia of the brachial plexus, it is interesting to recall that several investigators engaged in work on various forms of leukemia and on fowl paralysis have reported the absence of lesions in the nervous system. It has already been mentioned that Pappenheimer *et al* report the complete absence of lymphoid infiltration in the nervous system in certain cases. Then also Bayon[1] states that " simple lymphomatosis occurs frequently in fowls without any neural lesions." Warrack and Dalling[10] report that in the brains of only a small percentage of so-called normal birds have they found some slight evidence of the presence of the typical fowl paralysis type of cell. Findlay and Wright[3] also looked for infiltrations in the brain and state that " unlike Pappenheimer, Dunn and Cone (1927), who reported perivascular infiltrations in the brain of supposedly normal chicks, we have not found this change present in the brains of hens obtained from flocks with a negative history of fowl paralysis or epidemic blindness." Mathews and Walkey[5] report " In sixteen cases of lymphadenomas, derived from flocks in which there was no history of fowl paralysis, sections of the central and peripheral nervous system were found to be free of metastases." Also Richardson,[9] in his study of lymphatic leukemia, made a microscopic examination of the *sciatic nerves* of birds affected with this disease but showing no signs of paralysis to see if the nerves contained lymphoid tissue. In only one case did he find any lymphoid tissue present.

It would seem that in dealing with apparently normal birds or with birds affected with lymphomatosis, if the observations have been confined to the brain or sciatic nerves alone, it is not justifiable to draw conclusions concerning the presence or absence of lymphoid infiltrations. Thus in the case of six birds included in this study in which both the dorsal root ganglia and the sciatic nerves were examined, the former were affected but the latter were entirely free from lymphoid infiltration. Similarly, the brain was examined in four cases, and only one showed evidence of lymphoid cells and that very slight.

Althought the significance of slight lesions in the dorsal root ganglia of the brachial plexus of apparently normal birds can only be a matter of conjecture at present, yet it would appear that the constant occurrence of these lesions and also the fact that gross lesions are more apt to be found in this region would warrant a careful examination of this portion of the nervous system at all times. Furthermore, a histopathological and cytological examination of the dorsal root ganglia of the brachial plexus in cases of fowl paralysis and related diseases might help to throw some light on the nature and significance of the presence of lymphoid lesions in apparently normal birds.

References.

[1]Bayon, H. P. 1932. *Vet. Rec.*, **12,** 457.
[2]Biely, J., Palmer, V. E., and Lerner, I. M. 1933. *Can. J. Research*, **8,** 305.
[3]Findlay, G. M., and Wright, J. 1933. *J. Comp. Path. & Therap.*, **46,** 139.
[4]Jungherr, E. 1933. *Poultry Sci.*, **12,** 184.
[5]Mathews, F. P., and Walkey, F. L. 1929. *J. Cancer Research*, **13,** 383.
[6]Pappenheimer, A. M., Dunn, L. C., and Cone, V. 1929. *J. Exp. Med.*, **49,** 63.
[7]Pappenheimer, A. M., Dunn, L. C., and Seidlin, S. M. 1929. *J. Exp. Med.*, **49,** 87.
[8]Potter, J. S., and Richter, M. N. 1932. *Proc. Nat. Academy Sci.*, **18,** 298.
[9]Richardson, L. R. 1933. *Vet. Alumni Quart.*, **21,** 24.
[10]Warrack, G. H., and Dalling, T. 1932. *Vet. J.*, **88,** 28.

PRELIMINARY NOTE ON THE TRANSMISSION OF BOVINE HÆMORRHAGIC SEPTICÆMIA BY THE FLEA *CTENOCEPHALUS FELIS* BOUCHE.

By R. Daubney and J. R. Hudson,
Division of Veterinary Research, Kenya Colony,
and
J. I. Roberts,
Entomologist, Medical Research Laboratory, Kenya Colony.

Although the hypothesis that hæmorrhagic septicæmia of cattle and large ruminants is an insect-transmitted disease has often been tentatively advanced to explain how an organism so exacting in its *in vitro* requirements can survive from one outbreak of disease to another, there do not appear to be any records of attempts to transmit the disease with fleas.

During routine investigations of two recent outbreaks of hæmorrhagic septicæmia in cattle it was noticed that the younger cattle, particularly the unweaned calves, were heavily infested with fleas, *Ctenocephalus felis* Bouche. While adult cattle were dying from the subacute form of the disease on both these farms the heaviest mortality occurred in the unweaned calves. These calves were housed in rough wood and grass buildings with red earth floors, and the buildings were found to be harbouring large numbers of fleas, all of the one species.

At a later stage in the second outbreak fleas (average about 200 per animal) were collected from apparently healthy calves. By this time the disease had been responsible for the death of 20 calves, and the rest of the herd had been vaccinated twice with a killed vaccine. Fleas from 13 healthy calves were placed in tall battery jars and allowed to feed on healthy white mice. The mice were unsatisfactory as experimental animals, owing to the persistence with which they defended themselves against the attacks of the fleas. However, some fleas succeeded in obtaining a meal although on the whole they rarely managed to achieve full engorgement. In this experiment with roughly 2,600 fleas taken from healthy calves, only one positive result was obtained, a single mouse dying four days after infestation with fleas from Boran calf No. 127. The *post-mortem* findings in this mouse were consistent with a diagnosis of hæmorrhagic septicæmia, and bipolar bacilli

were present in smears made from the blood and organs. Presumably all the fleas had been eaten by the mouse since none could be found in the jar, and no larvæ merged from the litter which was kept under observation.

Four mice inoculated with a suspension of spleen tissue of the dead mouse died within four days and bipolar organisms were present in the blood stream. Cultures, recovered from the original flea-infected mouse and from the four inoculated mice, had the characters normal to *Pasteurella boviseptica*.

A few days later another calf, No. 21, died on the infected farm. This was next to the last calf to die in the outbreak, the only other death being that of a three-days-old calf that was born a month later and was not vaccinated. Fleas were collected from Calf No. 21 immediately after death, and were divided among eight individually caged mice. To confirm the diagnosis the blood of Calf No. 21 was inoculated into four mice, all of which were dead from hæmorrhagic septicæmia within 72 hours.

On the fourth day after infestation one of the eight flea-infested mice died and its *post-mortem* appearance was suggestive of a septicæmia with focal lesions in the liver. There were five adult fleas left in the jar. The blood of this mouse was inoculated to four mice, all of which died after the normal incubation period. All showed bipolar bacilli in the blood stream, and pure cultures were obtained.

A three months old calf, No. X9295, was inoculated into the prescapular gland with a suspension of blood from one of these four mice. The calf gave a temperature reaction on the following day and developed a large swelling at the site of inoculation. For three days the calf was very sick and during the first two days his clinical condition suggested that he might die. However, the swelling receded and he recovered completely. Five weeks later this calf and a control animal X9653, 15 months old, each received eight c.c. of a 24 hour culture of *P. boviseptica* recovered from mice inoculated with blood of the last calf to die in the outbreak, *i.e.*, the three-day-old already mentioned, which had been born subsequent to the vaccination. The culture was inoculated into the prescapular gland; in the case of X9295 into the gland opposite to the one used for the first inoculation.

The control died of the hyperacute form of the disease in less than 20 hours from the time of inoculation. Blood smears showed numerous bipolar organisms and pure cultures of *P. boviseptica* were obtained from the heart-blood. There was no marked local swelling but the inoculated lymphatic gland was infiltrated with clear straw-coloured exudate and the surrounding connective tissue was congested and hæmorrhagic. Calf X9295 showed a marked local swelling on the day after inoculation, and on this day refused to feed. On the following morning he was lame, but active and feeding. Apart from some local swelling, which persisted for

four days, he was perfectly fit from the morning of the second day onwards.

One can conclude that in both instances successful transmission to mice had been obtained with *C. felis*. Since the two mice had eaten all the fleas with the exception of five it cannot be decided how the transmission was effected.

It is not suggested for one moment that hæmorrhagic septicæmia of cattle is always flea-transmitted, but in tropical countries, where buildings are insanitary and often earth floored, one frequently finds on calves, sheep and goats enormous numbers of *C. felis*, a flea which these animals share with their native owners. And under such conditions, which are even encountered occasionally on European-owned farms, *e.g.*, in the two outbreaks under discussion, the part played by fleas in the transmission of the disease to young calves should not be discounted.

It is our intention to continue this investigation by direct experiments with fleas and calves.

A CASE OF SPONTANEOUS TUBERCULOSIS IN A GOAT.

By Herta Schwabacher.

London School of Hygiene and Tropical Medicine.

Griffith (1907) showed that the goat possessed an even greater susceptibility to inoculation tuberculosis than the calf. Spontaneous tuberculosis, however, although common in cattle, is very rarely observed in the goat. Thompson (1904) recorded that, in Saxony in the year 1894, out of 1,562 goats slaughtered 0·64 per cent. were tuberculous, and in Prussia in 1899 out of 47,705 goats slaughtered 0·41 per cent. were tuberculous. Melvin (1908), stated that only one goat was tuberculous out of 46,000 goats slaughtered under government inspection in the United States of America in the year 1908.

Koch (1884) described a case of spontaneous tuberculosis in a goat in which both lungs contained cavities as large as a man's fist. Isolated acid-fast bacilli were seen in films prepared from the cheesy pus that partly filled the cavities. Miliary tubercles were found in the lung tissue around the cavities, and tubercle bacilli were demonstrated microscopically within the giant cells. Large nodules were present in the spleen and liver, and the bronchial glands were greatly enlarged and caseous. No reference was made to the possible source of infection.

Slüys and Korevaar (1891), described a case of spontaneous tuberculosis in a fifteen months old goat which had been brought up on cow's milk. Generalised tuberculosis was found at necropsy. The primary lesions were considered to be in the alimentary canal, because the lesions in the intestines, mesenteric glands and liver appeared to be older than those in the other organs.

M'Fadyean (1892) considered that probably the first authentic case of spontaneous tuberculosis in a goat described in this country was that of Edgar (1892). The enlarged bronchial glands and tuberculous lungs of this three-year-old animal were sent by Edgar to M'Fadyean, who confirmed the diagnosis by histological examination. Typical giant cell systems and tubercle bacilli were found in sections prepared from the lung tissue. The source of infection was not ascertained.

Bulling (1896) described a case in which a six-year-old goat contracted tuberculosis from an unknown source. Eight other goats with which this diseased goat associated remained free from tuberculosis. The affected animal had been observed to cough occasionally over a period of two years. It was slaughtered on account of poor lactation, after having given birth to a healthy kid. At the post-mortem examination the lesions were found to be

confined to the thoracic cavity. Both lungs were adherent to the thoracic pleura. The upper lobes contained many small tubercles about the size of hemp seeds, which were situated along the bronchi. The lower lobes contained fewer and larger nodules, which were separated from one another by apparently healthy alveolar tissue. Some tubercles contained caseous material.

Griffith (1917) recorded the only case of spontaneous tuberculosis in a goat in which the type of tubercle bacilli has been determined. A six- to eight-month-old goat, which had been reared on cow's milk, was found to react positively to tuberculin, and was consequently slaughtered. About a dozen nodules were present in each lung, the lesions varying in size from millet seeds to large peas. The left bronchial gland and the long mediastinal gland were calcareo-caseous. A circular ulcer was found in a Peyer's patch of the small intestine, and the gland draining this ulcer was tuberculous. The pharyngeal gland and the spleen contained each a calcareo-caseous tubercle.

The growth in direct culture from a lung nodule was dysgonic, and in subculture was not improved by the addition of glycerine to the medium. Two rabbits inoculated subcutaneously, each with 10 mgm., died after 31 and 35 days respectively of severe generalised tuberculosis, identical with that set up by a tubercle bacillus of bovine type. Though infection in this case appears to have taken place by the alimentary route, it is interesting to note that the main lesions were found in the lungs.

History of the Present Case.

The goat was bought when it was seven days old from a poultry farm, where it had apparently been reared on its mother's milk. Subsequently it was fed on cow's milk, which " had been brought to the boil " and was reheated for feeding purposes. The hay which the animal was given was from meadows in which no cattle had grazed for the past eight years. The goat grazed with other goats until it died at the age of fourteen months.

Of the two kids born at the same time, one is still alive, the other died on the seventh day. No *post-mortem* examination on this kid was made.

Post-mortem Findings.

A fair amount of fluid was found in the body cavities and also extensive tuberculous involvement of the right lung, which was adherent to the thoracic wall.

The whole lung was extensively diseased, considerable portions of it being replaced by greyish-yellow caseous material. In the lower part of the apical lobe there was a cavity about 40 by 25 mm. containing a certain amount of softish breaking down caseous material. A similar cavity, but rather larger, about 65 by 65 mm., was found towards the hinder part of the diaphragmatic lobe. Throughout the remainder of the lung there were numerous

tubercles varying in appearance from the miliary to the large isolated type. In the apical lobe especially, tubercles, 4 or 5 mm. in diameter, had partly coalesced to form semi-caseous lobulated masses. Viewed from the surface the lung presented numerous large composite nodules raised slightly above the surrounding tissue, between which there were areas of more or less normal lung tissue. These, however, on closer inspection, were found to be riddled with tiny tubercles. Attached to the lung there was a tracheo-bronchial gland, 40 by 15 by 12 mm., almost completely converted into caseous material.

Histological Examination.

A section stained with hæmotoxylin and eosin shows pulmonary tissue containing two small nodules about one mm. in diameter, and one larger nodule about five mm. in diameter which has clearly been formed by the fusion of several small nodules. The nodules are composed chiefly of epithelioid cells. In some places the alveoli are distended with red blood corpuscles, lymphocytes, and epithelioid cells, and the alveolar septa are well defined. In other places all trace of alveolar structure is lost, and there are simply masses of closely packed epithelioid cells. In many areas, especially towards the centre of the nodules, the cell outlines of the epithelioid cells are indistinct and the nuclei poorly staining. The centre of the large nodule has undergone caseation. Only one giant cell of tuberculous type with peripheral nuclei is seen. There is some lymphocytic infiltration, especially towards the periphery of the nodules, and in the persisting alveolar septa in the substance of the nodules. The lung tissue, apart from that in the immediate neighbourhood of the nodules, appears normal. The histological appearances are those of a proliferative type of tuberculosis. The section stained for acid-fast bacilli shows the organisms in very small numbers in the caseous material, and in the epithelioid cells within the alveoli.

Bacteriological Investigation.

Isolation of the Strain.—An attempt to isolate the organism by direct cultivation from the lung tissue proved unsuccessful.

A portion of the lung containing several nodules was ground in a mortar and suspended in saline. Two rabbits and four guinea-pigs were inoculated with this material.

Rabbits.

One rabbit received 1·0 c.c. of the lung suspension intravenously and died after five days with no evidence of tuberculosis. The second rabbit inoculated intramuscularly into the left thigh with 1·0 c.c. was killed in a moribund condition after nine weeks. At the site of infection multiple small abscesses were seen. Each kidney contained a single small tubercle 2 by 2 by 2 mm. in diameter. Both lungs were extensively involved, each containing

about 50 caseous tubercles raised above the surface of the surrounding tissue. The spleen and liver appeared to be normal. Acid-fast bacilli were found in smears prepared from the kidney and lung, but not in the smears prepared from the splenic and liver tissues. It was concluded that the animal had died from a tubercle bacillus of bovine type.

Guinea-Pigs.

Guinea-pigs 849, 950, 852 and 853 received 0·3, 0·4, 0·5 and 0·5 c.c. respectively of the lung suspension intramuscularly. These animals when killed 38 to 49 days later all showed progressive generalised tuberculosis. Cultures of tubercle bacilli were obtained from the right sublumbar glands of guinea-pigs 849 and 850 and from the spleen of guinea-pigs 852 and 853. The splenic suspension in saline of guinea-pig 853 was inoculated intramuscularly into guinea-pigs 876 and 878, both of which died of progressive tuberculosis after eight and seven weeks respectively.

Investigation of the Strain 853.

A.—Cultural examination. Growth on Dorset egg was very poor and consisted of discrete slightly raised colonies with a rough surface and crenated edge. The Dorset egg cultures were inoculated on to tubes of egg and inspissated serum with and without glycerine, on to glycerine agar, and into five per cent. glycerine broth. Even after six weeks' incubation no growth was obtained on the glycerine agar medium or in the glycerine broth. Growth on the other media was dysgonic, and was not improved by the addition of glycerine.

B.—Biological examination. Five mg. and 10 mg. were inoculated intramuscularly into the left thigh of rabbits 339 and 341 respectively. These rabbits died after 38 and 35 days. Both animals had large local lesions extending along the whole shaft of the femur. There were miliary tubercles in the lungs, liver, spleen and kidneys, and in the peritoneum. Numerous acid-fast bacilli were seen in every field in films made from these organs.

From the dysgonic nature of its growth, its failure to respond to glycerine, and its ability to give rise to progressive generalised and fatal tuberculosis in guinea-pigs and rabbits, the strain must be regarded as of bovine type.

Summary and Conclusion.

1. A review is given of the literature referring to cases of spontaneous tuberculosis in goats.

2. Details are recorded of a fresh case, in which pathological and bacteriological examinations were carried out.

3. The tubercle bacillus isolated from this case was found to be of bovine type.

4. This appears to be the second case on record in which the type of the infecting bacillus has been determined. The previous

case, recorded by Griffith (1917), was also infected with the bovine type.

I desire to express my thanks to Professor R. T. Leiper who placed the lung at my disposal, to Dr. Creed for his opinion on the lung section, and to Professor Wilson for his advice on technical points and for his help in the preparation of this paper.

References.

Bulling. (1896). *Münch. Med. Wschr.*, **43,** 474.
Edgar. (1892). *J. Comp. Path. & Therap.*, **5,** 80.
Griffith, A. S. (1907). *Rept. Roy. Comm. Tuberculosis*, App. **1,** p. 47, p. 48.
——. (1917). *J. Path. & Bact.*, **21,** 342.
Koch. (1884). "The Etiology of Tuberculosis," The New Sydenham Society. (1886). **115,** 127.
M'Fadyean. (1892). *J. Comp. Path. & Therap.*, **5,** 61.
Melvin. (1908). 25th Ann. Rep. U.S. Bureau of Animal Industry, p. 98.
Slüys and Korevaar. (1891). *Ztschrift. für Fleisch u. Milchhygiene*, October. *Abstr. J. Comp. Path. & Therap.* (1891), **4,** 361.
Thompson. (1904). 21st Ann. Rep. U.S. Bureau of Animal Industry, p. 342.

A NOTE ON TWO NEW BLOOD PARASITES OF CATTLE, EPERYTHROZOON AND BARTONELLA.

By S. Adler
and
V. Ellenbogen.

(*Department of Parasitology, Hebrew University, Jerusalem.*)

During a study of infections of *Theileria annulata* we had occasion to observe several new blood parasites in cattle. They are of particular interest in so far as they represent genera which have up to the present never been observed in ruminants.

The name *Eperythrozoon coccoides* was applied by Schilling to a small parasite of the red cells which he and Dinger independently discovered in the blood of splenectomised mice. A detailed description of *E. coccoides* was given by Schilling (1928), Dinger (1929), and Lwoff and Vaucel (1931).

An Eperythrozoon was found in the blood of a splenectomised calf.

History.—Arab calf No. 21, about 9 months old, bought in Jerusalem, was inoculated with *T. annulata* on 14.4.34. The infection ran a mild course, and *T. annulata* appeared in the blood on 2.5.34. The infection was not accompanied by any marked anæmia and the red count varied between 5,000,000 and 5,600,000.

15.5.34. The animal was splenectomised.
16.5.34. The red count fell from 5,000,000 to 4,800,000.
18.5.34. A few red cells were found infected with *Eperythrozoon.*
20.5.34. 40 per cent. of the red cells were infected.
24 to 28.5.34. 90 per cent. of the red cells infected.
1.6.34. The infection disappeared.
24.5.34. Red count 4,350,000.
28.5.34. Red count 4,000,000.

The red count gradually rose to 4,800,000 on 6.6.34. Between 22.5.34 and 28.5.34, when the infection with the *Eperythrozoon* was at its height, the temperature varied between 40° and 40·6° C.

24.5.34. 110 c.c. blood from calf No. 21, inoculated into calf No. 11.

History.—13.1.34. Inoculated with *T. annulata*, which first appeared in the blood on 29.1.34. Parasites very numerous and red count fell to 2,100,000. As in all infections with *T. annulata*, the parasites persisted after clinical recovery.

22.4.34. The animal was splenectomised (red count at time of splenectomy, 5,500,000).

23.5.34. Red count, 5,200,000.

The Theileria infection was not influenced but a latent infection with *Anaplasma marginale* was activated as a result of splenectomy. *A. marginale* appeared in the blood on 29.4.34. The infection became very intense and as many as eight parasites were found in a single cell. A severe anæmia accompanied the infection of *Anaplasma* and the red count fell to 2,300,000 on 8.5.34. Subsequently the blood picture improved, the temperature was normal on 20.5.34, and the red count was 3,500,000 on 24.5.34.

30.5.34. *Eperythrozoon* first seen in a few red cells.
3.6.34. 40 per cent. of red cells infected.
4.6.34. 60 „ „ „
7.6.34. 90 „ „ „ and many parasites free in plasma.
8.6.34. 60 „ „ „
10.6.34. 15 „ „ „
12.6.34. Very few parasites in the blood.
14.6.34. No parasites found in blood smears.

During the course of the infection the temperature varied from 39·6 to 40·5° C.

Fig. 1. *Eperythrozoon wenyoni.*

Description of parasites.—(Fig. 1.) In general outline the parasite resembles *E. coccoides*. It is coccoid and often vesicular, stains a pale red with Giemsa (rather fainter than *E. coccoides*), and varies from about 0·2 to 1·5μ in diameter. Multiplication appears to be by budding and by fission. Up to 50 or 60 parasites may be found in one red cell, either scattered regularly throughout the cell, arranged in irregular chains or in tightly packed groups. They are also found on the periphery of the cell, of which they may occupy as much as two-thirds. The parasites at the periphery stain much more deeply than those in the interior of the cell, so much so that it was thought that we were dealing with a mixed infection, but this phenomenon occurs also in the case of *E. coccoides* in the mouse. Clusters of parasites were also found occasionally in large mononuclears.

We proposed the name *Eperythrozoon wenyoni* for the parasite described and dedicate it to Dr. C. M. Wenyon, F.R.S., of the Wellcome Bureau of Scientific Research.

Changes in the blood picture.—The most characteristic change in the blood picture is a striking anisocytosis, the red cells varying from 4 to 9 μ in diameter. This change was not found in four other animals splenectomised after an attack of Theileria. Apart from the anisocytosis there was some punctate basophilia and erythrophagocytosis. In the case of the second animal the blood findings are complicated by the presence of anaplasma, but in this case, too, the striking anisocytosis was probably due to *E. wenyoni*, for blood smears were almost normal a week before splenectomy.

It may be noted that uncomplicated *Theileria annulata* is not influenced by splenectomy. In four other animals which had recovered from *T. annulata* splenectomy performed up to 7 months after clinical recovery was not followed by a recrudescence of

clinical symptoms or by any noteworthy changes in the red cell count. The changes in the leucocytes were not constant, but in three of the animals there was a marked increase in both large and small lymphocytes and in large mononuclears. In two instances there was a large increase in the number of parasites and Koch bodies were found in blood smears (usually a bad prognostic sign in first attacks of Theileriasis), but this was not accompanied by anæmia or any clinical sign and the animals appeared normal in spite of the heavy blood infection.

It appears that there are two independent factors in acquired immunity to *T. annulata* ; a reduction in the number of parasites which persists indefinitely and a tolerance for the remaining parasites. The first factor may be temporarily absent and the increase in parasites may be such that at least 20 per cent. of the red cells are infected, without causing the animal the slightest inconvenience and without producing anæmia.

Bartonella sergenti n.sp.

Bartonella has been found up to the present in a large number of animals, including man, dog and several rodents, but has not been previously recorded from ruminants. We observed Bartonella in a calf inoculated with *Theileria annulata*.

Calf No. 7. 26.10.33. Inoculated with *T. annulata*. 8.11.33. Bartonella was found in red cells and persisted until 11.11.33. The number of infected red cells was less than 1 per 1,000. The parasites were bacilliform and up to 1 μ long (Fig. 2). From one to seven

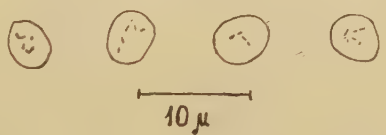

Fig. 2. *Bartonella sergenti*.

parasites were found in a single cell and their general appearance was similar to that of *Bartonella muris*. The appearance was not accompanied by any noteworthy symptoms, but apart from *Bartonella bacilliformis* none of the known species produce symptoms in non-splenectomised animals (unless their virulence has been artificially enhanced). We proposed the name *Bartonella sergenti* for the parasite of the cow and dedicate this species to Professor Edmund Sergent, of the Pasteur Institute of Algeria.

References.

Dinger, J. E. (1929). Naeheres ueber das Eperythrozoon coccoides. *Zentralbl. Bakt. Orig.*, Vol. cxiii, p. 503.

Lwoff, A., and Vaucel, M. (1931). Bartonelloses et infections mixtes. *Ann. Inst. Past.*, Vol. xlvi, p.258.

Schilling, V. (1928). Eperythrozoon coccoides, eine neue, durch Splenektomie aktivierbare Dauerinfektion bei der weissen Maus. *Klin. Woch.*, Vol. vii, No. 39, p. 1852.

ABSTRACTS.

NO-LESION CASES IN TUBERCULIN TESTING OF CATTLE IN THE UNITED STATES.

By E. G. Hastings, Walter Wisnicky, B. A. Beach and Jennet McCarter.

The tuberculin test in the United States was at first used mainly on herds in which the incidence of tuberculosis was high, as, for example, the pure-bred herds, and the number of animals tested was small compared to present-day numbers.

In the period from 1894 to 1908, 36,900 cattle were tested in Wisconsin, and of these 12·6 per cent. reacted. In the same State in recent years as many cattle have been tested in one week as were tested in that 14-year period. In the earlier period only selected herds were tested, while in the later period all herds have been tested. The test proved to be very accurate.

The testing of an infected herd, followed by the removal of the reactors and by one or two retests, in the great majority of cases sufficed to eliminate tuberculosis from the herd. Apparently the reacting animals showed lesions of tuberculosis in such a high percentage of cases that the question of the possibility that reactions might be due to other infections was not raised. When no lesions were found in reacting animals the case was always considered a positive one, with the disease either in the incipient stage or in the occult form.

During the two decades following the introduction of the tuberculin test students of bacteriology were united in their belief in the absolute specificity of micro-organisms, but within recent years some bacteriologists have come to speak of groups of organisms and to include in their consideration of the serum reactions the question of the so-called group reactions.

It is commonly admitted at present that organisms may be very closely related as far as antibody and anaphylactic reactions are concerned and yet be very different in their physiology and pathogenicity. A marked example of this is the avian tubercle bacillus and the bacillus of Johne's disease, organisms which are entirely different in pathogenicity, and certainly markedly different in their general physiology. The diagnostic agent made with one can be used apparently as successfully for the diagnosis of the disease produced by the other organism as it can for the detection of the disease produced by itself. [? Ed.]

The authors here cite the opinion of the late Dr. V. A. Moore, who stated that "as the testing of cattle became more common, it became more difficult to maintain the previous ideas regarding the specificity of the test."

As previously stated, the results of the tests in the period 1894 to 1908 seemed satisfactory in every respect.

In 1909, when compulsory testing of herds in Wisconsin was introduced, 48,000 cattle were tested; in 1910 207,000, and in 1911 32,000. The tests made in these years included, for the first time, the average run of herds in the country, and, as might have been expected, the percentage of reacting animals was markedly less than

in previous years when the pure-bred herds formed the major part of those examined. Of the animals tested from 1909 to 1912, 3·6 per cent. reacted to the test as compared with 12·6 per cent. in the previous work. In 1910, when the greatest number of animals was tested, the reactors found amounted to 3 per cent.

It was at this period that the no-lesion problem obtruded itself in Wisconsin. The percentage of reacting cattle that failed to show lesions of tuberculosis on slaughter was 17·2 per cent. in 1909, 21·8 per cent. in 1910, and 12·2 per cent. in 1911. It is to be noted that the highest percentage occurred in the year in which average herds made up a greater part of the total animals tested than in any other year. The explanation then advanced for the occurrence of no-lesion cases was, that a large part of the testing had been done by laymen. It was argued that if the testing had been done entirely by professional men the no-lesion problem would not have appeared. A study of the records, however, showed that the no-lesion case was not connected with the type of tester but apparently did have a direct relation to the type of herd tested. In those herds in which the incidence of tuberculosis was low a high percentage of the reacting cattle failed to show lesions (see table).

Animals Removed from Herd.	*Herds from which Animals were removed.*	*Animals Killed.*	*No-Lesion Animals.*	
			No.	*Per cent.*
1	1063	1063	468	44·0
2	346	692	248	35·8
3 or 4	210	699	18	26·9
5 to 9	207	1167	135	13·1
Above 10	120	2105	145	6·8

The experience of veterinarians and of students of bovine tuberculosis led them to believe that no other animal other than a tuberculous one would react to tuberculin. The comparatively few reacting animals which failed to show lesions of the disease on slaughter, and the consistent success with which herds were freed from tuberculosis by use of the test, were thought to justify belief in its absolute specificity. No one was thinking of the no-lesion problem as it exists to-day.

The introduction of the intradermal test made possible the examination of great numbers of animals at small expense. It led to the area method of testing cattle, in which the average run of herd has been the unit examined under these conditions. The no-lesion case has become as important in some other parts of the country as it was in Wisconsin in 1910.

As soon as testing became general it became evident to the students of bovine tuberculosis that the accuracy of the test should be expressed in terms of animals tested rather than of animals reacting, for otherwise the no-lesion case would make an alarming percentage of the cattle slaughtered, and its expression in terms of reacting cattle would lead to unwarranted conclusions by the cattle owners.

Previous to the general testing of cattle the skin lesion was practically unknown, but it was found in a considerable part of the reacting cattle which came to be slaughtered after the test became general. It offered another source of relief from the trouble which

the no-lesion case presented. There was reason to believe that the skin lesions were tuberculous in nature, for they almost constantly revealed acid-fast organisms. The mass of evidence which has been accumulated during the past few years, however, would indicate that the skin lesion is not tuberculous in origin, but is apparently due to some other member or members of the group of mycobacteria.

The no-lesion case seems to vary widely in incidence in different sections of the country. In some areas it is still claimed that practically all reacting cattle show lesions of tuberculosis. The most extreme example of the no-lesion case is that supplied by the representative of the Federal Bureau of Animal Industry in Utah, in which out of 3,323 reacting animals only 254 revealed internal lesions of tuberculosis, while the remainder showed skin lesions, or no detectable evidence of tuberculosis.

The no-lesion case has gradually forced its recognition on a hostile audience, until it is believed that there are few areas in which great numbers of tests are being made in which the problem is not now deemed worthy of consideration by the Live Stock Sanitarians.

If one admits the possibility of sensitisation to tuberculin by any other cause than the bovine tubercle bacillus he is likely to turn to one of the other types, the human or avian, as the probable cause, and it is thought probable that each of these may in some cases be responsible. Saprophytic organisms of the tubercle group present another possible explanation for the non-specific sensitisation. Two of the present authors in a previous paper described experiments on sensitisation to tuberculin in cattle by the injection of acid-fast bacilli, other than tubercle bacilli, isolated from the tissues of no-lesion reacting cattle.

The expression " no-lesion " is preferred to the more common " no visible lesion." The implication of the latter term is that every such animal is a tuberculous one, of the former that some of them may be free from tuberculosis or at least from any tubercle bacillus which needs to be considered from the standpoint of the continued health of the herd. The authors think that the observations here referred to should be taken as indicating the possibility, not the probability, that organisms similar to those studied may be responsible for some part of the no-lesion cases.

There is held to be a real possibility of sensitisation to tuberculin by bacteria other than the mycobacteria. Holth, in Norway, where the no-lesion problem is also encountered, has stated that the tuberculin tests discovers more tuberculosis in the cattle than exists, and he has recently mentioned the possible relation of Bang's disease or contagious abortion of cattle to the tuberculin test. He has found that guinea-pigs inoculated with *Br. abortus* sometimes react to tuberculin. He tested the cattle of some tuberculosis-free herds which were infected with abortion and obtained some positive reactions to tuberculin which appeared to be due to infectious abortion itself.

The present authors are of the opinion that a wealth of data in support of the validity of this suggestion could soon be collected in the United States. It is believed that one of the deficiencies of the tuberculosis eradication problem has been the lack of study of collected data. A huge pile has been collected in the Records of State and Federal Agencies, but almost no effort has been made to draw some useful knowledge from it by study.

Originally the authors thought that some change in the technique of the test, or in the diagnostic agent, might help to avoid whatever error exists in the present procedure, but it soon became evident that this was a faint hope, and that while the test itself could not be modified the interpretation of the results might, and that, instead of basing the conclusion as to the presence or absence of tuberculosis in the herd on the test alone, the herd-history might be invoked to aid. As the counties are tested and retested a history, official and private, of each herd accumulates. If a herd has a negative history as regards bovine tuberculosis over a number of years, a reaction in one animal of the herd has a different import from a reaction in a herd with a history of tuberculosis. Objection may be made that there are many ways in which the bacillus may enter a herd, but, as the areas brought under control become continuous and extended, the danger of the reinfection of the herds becomes less, and especially in such a territory as is represented by the major part of Wisconsin, from which cattle are constantly passing out and almost none are passing in.

The Control Official is in constant fear of breaks, with the resulting injury to the prestige of this work, especially in removing territory from the modified accredited area. The slow spread of tuberculosis from herd to herd in a great part of the United States in which the condition is similar to that occurring in much of Wisconsin, is indicated by the data of Wisconsin. In the areas in which the herds were relatively static the incidence of tuberculosis was found to be low on the initial area test in spite of a long period, 40 years or more, of intensive dairying. Such data indicate that the spread of tuberculosis from herd to herd is slow in areas in which the herds are largely static, and more rapid in areas in which constant buying is taking place. They indicate the possibility of using a procedure in one section which might be inadvisable in another.

In order to test the relative safety of taking the history of the herd into consideration to supplement the tuberculin test in determining the condition of the herd, the following work was carried out.

It had been noted that the no-lesion cases made up a larger part of the group of reacting animals which consisted of herds with only one reactor in each, than of the groups in which there were two or more reactors per herd. It was decided to begin work with the one-reactor-per-herd group. The history of each herd was obtained largely from the owner, whose recollection of the results of the tests of his herd was usually vivid and correct. The official records also served as a source of information. It appeared from experience that a reasonably complete history can be unearthed in areas in which dairying is intensive, or in those areas in which the herd is the chief concern of the owner rather than a side issue to some other branch of farm work.

In the second complete or general test of a county the inspectors were requested to report immediately those herds in which only one animal had reacted. A visit was paid to a number of such farms in order to learn the history of the herd, and 15 herds were selected for investigation, each owner being requested to allow the animal that had reacted to remain in the herd for subsequent retesting, and for removal and eventual slaughter by the authors, irrespective of the results of the retests. The results with regard to these animals are shown in the following table:—

Herd and Animal.	*Age.*	*Subcutaneous Reaction.*			
		Intradermal Reaction.	*Maximum Pre-injection Temperature.*	*Maximum Post-injection Temperature.*	*Lesions on Slaughter.*
1	10	Negative	100·8	105	Nodules, supra-mammary gland
2	6	Negative	101·6	102·4	None
3	3	Negative	102·2	105·4	None
4	7	Negative	102	105·2	Skin
5	4	Negative	102·2	101·6	None
6	3	Negative	101·6	103·4	None
7	3	Negative	102·4	103	None
8	7	Negative	102·2	102	None
9	3	Negative	101·8	103·6	None
10	3	Negative	101·6	104	Skin
11	3	Negative	102·2	102·8	None
12	3	Negative	102	104·4	Skin
13	2	Negative	102·4	102·4	None
14	6	Negative	101·4	102·2	Skin
15	4	Negative	101·6	104	None

The retests were made about 90 days following the one in which the reactors were found. The animals were killed at an establishment maintaining Federal inspection, and were examined in greater detail than usual for the detection of abnormalities; suspensions of material from the cervical, thoracic, and mesenteric lymphatic glands were removed from each animal and used for the inoculation of guinea-pigs, rabbits and fowls, and also with the object of obtaining cultures after treatment with 50 per cent. antiformin for 20 minutes.

As the table shows, suspicious lesions were found in only one (No. 1) of the 15 animals, and the results of the experimental and cultural tests of the lymphatic glands were negative in every instance, with the exception that an acid-fast organism, which was proved to be other than a tubercle bacillus, was obtained from animal 3, which was one of those giving a positive reaction to the thermal tuberculin retest.

A retest of the herds from which the animals came six months after the test in which the reactors were found revealed no reactors.

The results of inoculation experiments were as follows:—

Rabbits.—Negative results.

Chickens.—Negative results.

Guinea-pigs.—1, died after four months with subcutaneous lesions, and also lesions in liver and spleen.

2, killed after five months. One large caseous lesion in the liver. Many acid-fast rods found in the lesion.

3, subcutaneous lesion only.

4, subcutaneous lesion only.

No growth could be obtained from guinea-pig 2, and inoculation of two guinea-pigs and two fowls from guinea-pig 2 yielded only negative results. One rabbit inoculated died after four months, and in it no changes were found other than an enlarged spleen, in which nothing could be found microscopically.

The nodules found in the supramammary gland of the bovine No. 1 were neither caseous nor calcified, and were not considered typically tuberculous in appearance. The authors think that it is justifiable to

conclude that the animal was not tuberculous in spite of the positive reaction to the retest by the subcutaneous method.

It is to be noted from the table that the intradermal retest was negative in all the animals, but the subcutaneous was positive in six out of 15 shown in the following table.

Animal.	*Maximum Post-injection Temperature.*	*Lesions on Slaughter.*
1	105·0	Supramammary lymph-nodes (?)
3	105·4	None
4	105·2	Skin
10	104·0	Skin
12	104·4	Skin
15	104·0	None

Animal 14 showed a skin lesion but had no temperature response.

A second group was selected for investigation from herds undergoing a second complete test in another county, the herds in question having a history of freedom from tuberculosis.

In this case the animals were removed from the several farms to an experiment station, as it was intended to make a more detailed study of each case than would have been possible if the animals had been allowed to remain in their respective herds.

The previous history of the animals forming this second group are shown in the table.

Animal	*Previous Herd Tests.*	*Results of Previous Tests.*	*Condition of Poultry.*
1	11	No reactors	Unknown
2	1924, 1925, 1926, 1928	2 reactors in 1925	Healthy
3	1928	No reactors	Tuberculous
4	4	No reactors	Tuberculous
5	1928	No reactors	Healthy
6	1928	No reactors	Possibly tuberculous
7	1928	No reactors	Tuberculous
8	1928	No reactors	Healthy
9	1928	No reactors	Tuberculous
10	1928	No reactors	Tuberculous
11	4	No reactors	Healthy
12	4	No reactors	Healthy
13	1928	No reactors	Tuberculous
14	1928	No reactors	Healthy

The two reactors found in 1925 in the herd from which animal 2 came showed no lesions of tuberculosis on slaughter. Animals 11 and 12 came from the same herd.

Animal 5, a male, was tested once and was slaughtered soon after the group was formed. Animal 6 died before more than one retest was made. The tests were negative in both instances, as were also the *post-mortem* examinations.

Since the poultry on part of the farms was tuberculous it was thought

advisable to retest with avian tuberculin and also to give double the dose of each tuberculin used.

Each of the animals in this group was retested three times by the intradermal with mammalian tuberculin, and three times intradermally with avian tuberculin.

With one exception (No. 1), each of the twelve animals gave a positive response to one or more of the various tests made at the first retesting period. At the second test period six reacted, and at the third test period none.

Ten tests were made on each animal with mammalian tuberculin and six tests with avian. Only one animal gave consistent responses to either tuberculin, and the exceptional animal was from a herd which had long been on the accredited list. Out of ten chances to react to mammalian tuberculin a positive result was noted in six cases and a suspicious result in two cases. Out of six chances to react to avian tuberculin there was only one positive result.

The authors think that if one had to hazard a guess as to the cause of the sensitisation of these animals from the data presented it would be that avian bacilli had something to do with it, since on eight of the eleven farms there was apparently opportunity for such a cause to be operative. The greater number of positive responses to avian than to mammalian tuberculin is cited as evidence for this theory.

The authors observe that it would seem that the eradication of avian tuberculosis may be necessary in any area in order to ensure that no reactions will be obtained in cattle injected with mammalian tuberculin. It is also considered possible that human tubercle bacilli may now and then be responsible for the sensitisation of cattle to tuberculin.

A subsequent test of the herds from which the above second group of animals came six months after the original test showed no reactors.

The reacting animals of the second group were slaughtered soon after the last tests were completed. Skin lesions were found in two animals, Nos. 4 and 12 in the list. Animals 1, 3, 7, 8, 9, 10, 11 and 14 were negative as to lesions. The search for evidence of infection in these animals was carried out in the same way as with the first group, that is, it included the inoculation of guinea-pigs, rabbits and fowls with suspensions of material from the lymphatic glands. From the lymph nodes of one animal (No. 9) an acid-fast organism was found and proved by cultural and experimental inoculation to be an avian tubercle bacillus.

In animal 2 a lesion caused by the *Actinomyces bovis* was found in the retropharyngeal glands.

Lesions of tuberculosis were found in the lungs and in the cervical, bronchial and mediastinal glands of animal 13, and guinea-pigs and rabbits inoculated from this animal showed frank tuberculosis with lesions from which a culture of the mammalian tubercle bacillus was obtained. A retest of the herd from which this animal came ten months after the original test revealed three reactors, one of which showed a lesion in a bronchial gland and the other two lesions in the mesenteric glands. In the previous test, six months after the original test, there had been no reactors.

It was subsequently discovered that in this herd there had been at least two possible opportunities for infection, (1) through the purchase of a bull from an infected herd, and (2) by association at pasture of three animals, the identity of which could not be established, with a herd found to be almost 100 per cent. tuberculous.

SUMMARY.

The detailed study of two groups of cattle consisting of one-reactor-per-herd animals indicates that some other organism or organisms than the bovine tubercle bacillus may sensitise cattle to tuberculin.

The organisms to which consideration should be given in this connection are the avian and the human tubercle bacilli, possibly Johne's bacillus, and the saprophytic members of the tubercle group of bacteria.

It is believed that the observations herein recorded indicate that the herd-history may, in the final stages of the eradication of bovine tuberculosis, serve as a supplement to the tuberculin test. It may prove possible to avoid the removal of a considerable part of those animals which now form the no-lesion group without endangering the eradication work in any way, by allowing herd history to influence the judgment as to the condition of the herd as regards tuberculosis. (*Journal of the American Veterinary Medical Association,* Vol. LXXXII, 1933, pp. 565-583.)

EQUINE ENCEPHALOMYELITIS.

By EDWARD RECORDS and LYMAN R. VAWTER.

History.—During the summer of 1930 the disease appeared among horses and mules in San Joaquin in California, and investigations made by Meyer, Haring and Howitt of the University of California revealed that it was caused by a filtrable virus.

During 1931 the disease appeared in different parts of Nevada, the first case occurring about the middle of July, and others continued to occur in large numbers until the advent of cool weather about October 1st. No connection was established between the California and Nevada outbreaks. The disease recurred in Nevada during the summer of 1932, appearing first late in June.

During the epidemic season of 1932 the disease not only recurred on some of the ranches where it appeared in 1931, but spread to adjacent and outlying districts. Every county in Nevada except one reported major or scattered outbreaks. The main epidemic area was in Western and North-Central Nevada, where the horse population was concentrated during the harvesting and haying seasons.

Old residents reported two earlier epidemics of a similar nature in Nevada, one during the late nineties of the past century and the other about 1910, but sporadic cases and minor local outbreaks are believed to have occurred in Nevada for at least 25 years. These minor outbreaks occurred at all seasons of the year, and, although localised, the death rate was sometimes very high. In some instances practically all the horses on a ranch died.

The present authors confirmed the discovery by Meyer and others that the causative agent is a filtrable virus with neurotropic properties.

Seven strains of virus from different localities in Nevada were studied experimentally. They all produced encephalomyelitis in horses and guinea-pigs. All the strains passed through Berkefeld N. filters and they were all serologically identical. Antiviral serum prepared with a strain from one district protected guinea-pigs against the other strains obtained from different localities in Nevada.

Encephalomyelitis of equines is an acute infectious, and probably contagious, disease of horses and mules. It is characterised by depression or lagging at work and high fever in the early stages, with subsequent paresis, inco-ordination, prostration, motor and sensory paralysis, appearing rapidly in the order given. Psychic disturbances and impaired vision or total blindness are observed in some cases. The mortality rate is high. Animals that survive an attack of the disease frequently suffer permanent disability, such as motor ataxia, lack of endurance, or blindness, or become permanent "dummies."

Very little is known regarding the natural transmission of the disease. Mosquitoes and biting flies have been suspected because the disease becomes epidemic in the summer months when these insects are active. Kelser claims to have transmitted the disease from guinea-pigs to a horse through mosquitoes, but the authors think that that theory is not wholly tenable under Nevada conditions, as some of the localised outbreaks there with a high mortality have occurred in the winter months.

The fact that susceptible horses readily develop the disease following intranasal instillation of virus appears to indicate that the upper respiratory tract might not only be a favourable point of entry for the virus but also harbour the virus and create carriers.

Apparently corrals, feeding racks, or feeding troughs did not appear to act as vectors of infection. Many new horses were placed in a corral in which 25 horses injected with virus had developed typical symptoms of the disease and either died or were destroyed, without any of them contracting the disease. All of the presumably exposed horses proved susceptible to either intracranial or intranasal administration of virus one to four months after arrival. Furthermore, the location of the corrals was such that mosquitoes and biting flies were relatively plentiful during the seasons of the year when these insects are active. There were no survivors among horses which developed encephalomyelitis as a result of intracerebral or intranasal injection of virus to act as carriers in this group, but horses which recovered from natural attacks of the disease elsewhere were added to serve in that capacity.

New horse stock brought to premises having a history of encephalomyelitis in recent months may develop the disease in a week or ten days after arrival.

The practice of interchange of horses from one ranch to another during the harvesting or haying season is suspected to be one way of spreading infection.

There appears to be considerable variation in the type of clinical cases observed even in the same locality, and it is suspected that this may be due to variations in virulence of the virus, or to the natural resistance of the individual horse.

The following types have been recognised:—

The sleepy or ambulant type shows high fever, depression and cessation of peristalsis, lip droop, muscular twitching and even dysphagia, but does not exhibit any serious disturbance of motility. This type may be given a favourable prognosis.

The paralytic type may be regarded as a further progression of the sleepy type, but develops paresis and inco-ordination and may need a sling or supporting frame to keep it on its feet. A guarded prognosis should be given this type of case.

The apoplectic or bulbar type appears suddenly, has a rapid course, and after a short period of inco-ordination, with cardiac and respiratory distress, is followed by death in six to twelve hours. Treatment has always failed in this type of case.

The violent or excited type has occurred in about 5 per cent. of Nevada cases. In several violent cases vision was impaired, which caused stumbling and accounted for breaking through fences and running into other objects, this in turn further exciting the subject.

Clinical Symptoms and Course.—From clinical and experimental studies made during the past two years it appears that the disease generally has three stages. Many individuals requiring clinical attention never develop any serious motor derangement or reach the prostration stage. It is believed that many benign cases also occur during an epidemic and escape notice.

The first or incipient stage is attended by high fever, 104° to 107° F., reddish icteric colouration of the conjunctiva, and a variable degree of depression. Cessation of peristalsis and moderate constipation may be present. Tenesmus is frequently observed during defæcation.

The second stage usually follows in 18 to 36 hours and the temperature drops to from 101° to 104° F. It is in this stage that disturbances of motility, such as paresis and inco-ordination, begin to appear. One or both lips may droop and wrinkle, accompanied by twitching of the facial muscles and lips. Twitching of the muscles of the shoulder and the neck also occurs. There is little desire for food or drink and a marked decrease in weight occurs in 24 hours. Inability to swallow food or liquids is a frequent symptom. Nasal discharge appears in many individuals during this stage and the mouth odour is always extremely fœtid.

Pointing one fore foot or dragging the hind feet, backing up, and a weaving gait may also occur. The subject frequently backs or pivots around until it can reach a building or fence against which to brace itself. A base-wide position of the front legs and holding the head down are characteristic poses. Extension of the penis occurs frequently in stallions and geldings.

Bilateral or unilateral impairment of vision or total blindness may be present. Sensory paralysis sometimes begins late in the second stage, following an irregular pattern over the hips, back and hind legs, although it may not appear until the prostration stage. The veterinarian usually does not see the case until this second stage is reached, especially in districts where the disease has not previously occurred.

The third stage is that of prostration, when the subject collapses and is no longer able to get up with assistance or even stand up with support, due to generalised motor paresis. Horses at pasture often reach this stage before the owners are aware of any sickness. During the early part of the prostration stage the subject struggles violently, digging deep holes in the ground or wrecking stalls and mangers with the paddling movement of the feet. Clonic spasms of a few seconds' duration may occur at irregular intervals, particularly upon handling.

The pulse and respiration are accelerated, later becoming arythmic. The temperature may be either slightly above normal or even subnormal, depending on the period of prostration. Sensory paralysis, if not already present in some parts, begins to appear in an irregular

pattern, in the loins, hips, flanks and legs, gradually spreading forward. In many cases the skin over the trunk and legs becomes totally insensitive while the head and neck region are hypersensitive. The fæces are retained and the anus may be flaccid or even patulous. The bladder is usually paralysed later in the prostration stage and the urine becomes syrupy. Some individuals manifest flaccid motor paralysis before death.

As a rule the subject passes through the three stages in three to four days, death occurring after twelve to 36 hours' prostration. Some individuals may live several days in a prostrate condition but they become pitiful sights, due to bruising and decubitus, or die from secondary pneumonia.

The prognosis is most unfavourable when the prostration stage is reached, although a few subjects recover with careful nursing, but the motor functions are usually permanently impaired. Those showing profound prostration may be considered hopeless and be destroyed for humane reasons.

Differential Diagnosis.—Apparently equine encephalomyelitis has been repeatedly confused with botulism. The essential condition of the latter disease is the presence of toxin in the food. In outbreaks of botulism several animals that have had the same food are generally infected, whereas encephalomyelitis usually affects only one or possibly two horses at a time without any direct food connection. The appearance of similar cases on separate ranches in a community without any food connection should be regarded with suspicion.

Post-mortem examination of horses suspected of having died from the disease is advisable though no pathognomonic lesions other than generalised icterus and increased spinal fluid may be found. Such an examination coupled with clinical history and symptoms may be of great value in arriving at a diagnosis by excluding other conditions which might have caused death.

Treatment.—Animals showing suggestive symptoms of encephalomyelitis should be retired from work at once and moved slowly to a cool, shady and quiet place if possible. One attendant should have care of the affected animals, and other persons or animals should be kept away. In the day time, during hot weather, the entire body and head may be bathed with cold water at frequent intervals or the body and neck covered with a blanket kept saturated with cold water. Ice packs may also be used on the back, shoulders and head. In regions where low night temperatures occur in the summer time the body should be dried off by nightfall to avoid chilling and secondary pneumonia. For cases occurring during cold or stormy weather this method of handling must, of course, be modified in accord with temperature or climatic conditions. Strong draughts and actual chilling are always to be avoided, as these cases are rather prone to develop pneumonia.

Large enemas of cool water, using three to six gallons every six hours, not only remove the stagnating contents of the lower bowel but also reduce body temperature, exert a definite stimulating effect on the patient, and may help to maintain the body fluids so essential in these cases. Some individuals can swallow small amounts of water if it is given slowly. The mouth may be irrigated frequently to keep

it fresh and clean. The stomach tube may be used to administer liquids, but not on subjects having a profuse nasal discharge. Drenching with liquids is dangerous.

It is a good plan to make a supporting frame for horses that are likely to collapse. A sling may be used in conjunction with the supporting frame, but one should never depend upon a sling alone. The tendency is to break back or lean against some object, and the supporting frame, or combination of frame and sling, supplies this need. If the patient is already down and cannot or will not get up, but can still rest on its sternum, padding with sacks of straw or sand is advisable. The patient should not be allowed to lie for any length of time in a prone position.

Drug treatment has not proved entirely satisfactory. The removal of the stagnating contents of the lower bowel is desirable if it can be accomplished early in the attack. Drastic purgatives such as eserine or arecoline should be used cautiously or avoided entirely, as they are liable to cause violent cramping and collapse in subjects already well advanced in the second stage when motor derangement and incoordination or staggering are present.

Prior to 1932 the only treatment available was nursing and drug therapy, the greatest dependence being placed on nursing. Serum from convalescent or recovered cases of encephalomyelitis failed to show clinical value. During 1932 the Nevada Agricultural Experiment Station was successful in producing a highly potent antiviral serum by the hyperimmunisation of horses with encephalomyelitis virus.

Immunity.—It was found that horses from ranches or districts having a recent history of encephalomyelitis are likely to be immune to intracerebral injections of virus, even though they were not known to have an attack of the disease. It therefore appears likely that equine encephalomyelitis is a pandemic disease involving practically all of the horse population in an epidemic area, but only a certain percentage develop definite clinical symptoms.

The disease can recur in the same individual; this is substantiated by the report of four such instances in the past two years.

When a diagnosis of encephalomyelitis has been made in a given group of horses, if possible daily temperatures of all horses and mules should be taken. High temperature and conjunctival congestion often appear 24-48 hours before any other positive clinical signs are present.

Veterinarians and attendants should be careful when handling encephalomyelitis cases, as three instances are reported of men contracting this disease, one of them being a veterinarian. (*Bulletin No.* 132, *University of Nevada*, 1933.)

CONTENTS

GENERAL ARTICLES.

PAGE

The Epidemiology of Winter Outbreaks of Parasitic Gastritis in Sheep, with Special Reference to Outbreaks which occurred during the Winter of 1933-34. By E. L. Taylor ... 235

Pulpy Kidney Disease (Entero-Toxæmia) of Sheep in Palestine. By S. J. Gilbert 255

Foot-and-Mouth Disease : The Fixed Virus Types. By R. Daubney 259

A Study on " Cell-Inclusion Disease " in Fowls :—

I. On the Identity of Acute Cell-Inclusion Disease and Fowl-Plague 282

II. On the Diagnostic Value of the " Chromatic-Inclusions " in the Leucocyte. By A. Komarov 296

The Study of Avian Biology in Relation to Scientific Progress. By H. P. Bayon 302

The Incidence of Tuberculosis among Cattle belonging to the Wachagga Tribe in Tanganyika Territory (E. Africa). By R. L. Cornell 320

OBITUARY.

Professor S. H. Gaiger 330

NOTICE TO SUBSCRIBERS.

Communications, exchange journals, specimens, etc., for the Editor should be addressed to Sir John M'Fadyean, Highlands House, Leatherhead, Surrey.

BINDING CASES.—Maroon Full Bound Cloth. Can be obtained from the Publishers and Agents, Price 3s. 6d., post free.

ELIXIR HORMONIGEN

(HEWLETT'S)

A powerful and palatable preparation of the Hormones:—Thyroid, Pituitary, Ovary and Testes, constituting a valuable remedy for Bronchitis, Asthma, Chorea, etc., in Dogs.

This preparation has also been recommended for Rickets and Arthritis in young dogs and particularly in the treatment of Skin Diseases. Many of these, such as Eczema, are largely the result of glandular deficiencies, and Elixir Hormonigen leads to great improvement—often curing forms of this disease that have resisted all other remedies.

It is also extremely useful as a general tonic.

Capt.———, M.R.C.V.S., has had great success in the treatment of Bronchitis and Asthma, the animal being cured in one month. A paralysed Terrier, that could not stand or raise the head and had no co-ordination of its limbs, was treated with Elixir Hormonigen and made a rapid and perfect recovery.

"I have had most satisfactory results in using this elixir in cases of chorea, and allied nervous complaints occurring as a sequel to so-called distemper; it has a most surprising and definite curative effect in even advanced and chronic cases." J. F. T. ———, M.R.C.V.S.

"In dogs and cats I use your Elixir Hormonigen as a tonic after all debilitating diseases and fevers. It is palatable, appetising and works wonders." E. J. L. ———, M.R.C.V.S.

The dose for Terriers, etc., is one teaspoonful 3 or 4 times a day **before** food. Airedales may be given a dessertspoonful and larger dogs in proportion. Puppies, up to 6 months, half the above doses.

Price: 6/6 per lb.; W. Qts., 6/- per lb.

Also in bottles, labelled "TONIC ELIXIR FOR DOGS," with dose,
2 oz., 15/-; 4 oz., 25/- per doz.

Hormonigen Tablets

(HEWLETT'S)

Containing various pluriglandular extracts that have been found useful in Veterinary practice.

Hormonigen No. 1, for paralysis in dogs, 3/3 per 100; 30/- per 1,000. For sterility in cattle, particularly where œstrum is absent, 60-grain tablets, in tubes of 10 tablets, 1/6 per tube.

Hormonigen No. 5, for sterility in bitches, 3/6 per 100.

Hormonigen No. 6, for sterility in dogs, 2/6 per 100; double strength, 4/6 per 100.

Prepared only by

C. J. HEWLETT & SON, LTD.

WHOLESALE VETERINARY DRUGGISTS,
35-42, Charlotte Street & 83-85, Curtain Road, London, E.C.2.

AGENTS FOR THE VACCINES AND SERA OF THE BACTERIOLOGICAL INSTITUTE, TOURS.

Tuberculin (Subcutaneous, Intradermal and Ophthalmic), Mallein, Vaccines for Canine Distemper (Prophylactic and Curative), Pneumonia, Strangles in Horses, and Mastitis, etc. Pyovaccine for Sores and Contagious Lymphangitis.
Also Agents for the Jensen-Salsbery Biological Products, Pasteur Institute, Paris, etc.

Circular regarding Vaccines, Sera, Aggressins and Bacterins, sent free on request.

Only supplied to Members of the Veterinary Profession.